JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VII WASHINGTON, D. C., OCTOBER 16, 1916 NO. 3

EFFECTS OF NICOTINE AS AN INSECTICIDE

By N. E. McINDOO [1]

Insect Physiologist, Deciduous Fruit Insect Investigations, Bureau of Entomology

INTRODUCTION AND EXPERIMENTAL METHODS

The pharmacological effect of nicotine ($C_{10}H_{14}N_2$) on the higher animals is well understood, but there is practically nothing known about the pharmacological effects of nicotine as an insecticide. Owing to the high cost of nicotine, it is desirable to have a substitute for this insecticide. Before being able to discover, if possible, such a substitute, it is first necessary to ascertain how nicotine affects insects.

In the investigation herein recorded two chief objects have been kept in view: (1) To determine the physiological effects of nicotine as an insecticide, and (2) to trace the nicotine into the insects after it has been applied to them. A brief account of the pharmacological effects of nicotine on other animals and the views pertaining to the physical and chemical effects of nicotine on the cells are also given.

Owing to the small size of the insects utilized in the experiments the usual method of procedure employed by pharmacologists could not be used, because it was impossible to operate on living insects in order to ascertain what tissue is vitally affected by nicotine. Consequently the behavior of the insects treated with nicotine was compared with the behavior of normal and untreated ones; and immediately after the treated ones had died, they were fixed in a fluid containing a nicotine precipitant. By this means the nicotine was precipitated wherever it had gone into the insects; and after making microscopical sections from these insects, it was not a difficult task to trace the precipitated nicotine.

Shafer (20, 21),[2] from the standpoint of a physiological chemist, carried on investigations to determine how contact insecticides kill. He did not

[1] The writer is grateful to the following persons: To Mr. A. F. Sievers, Chemical Biologist in Drug-Plant and Poisonous-Plant Investigations, for extracting pure nicotine from a commercial nicotine material and for verifying the percentage of nicotine in a sample of 40 per cent nicotine sulphate; to Dr. D. E. Jackson, of the Department of Pharmacology, Washington University Medical School; and to Mr. O. D. Swett, Assistant Professor of Chemistry in George Washington University, for reading and criticizing the manuscript of this paper.

[2] Reference is made to Literature cited, pp. 120-121.

experiment much with nicotine and did not endeavor to ascertain what tissue is vitally affected by any particular insecticide, but he seems to infer that nicotine affects the cells chemically in the same way as do the other contact insecticides. Even if nicotine has no therapeutic use, it is classified as a poisonous drug; and for this reason the investigations in which it is used give the best results when it is considered from the standpoint of toxicology.

PHYSIOLOGICAL EFFECTS OF NICOTINE

At the outset it was decided to select one of the most specialized insects and to feed it nicotine so that the results might be compared to those previously obtained after administering nicotine to certain higher animals. Although nicotine as an insecticide is rarely used as a stomach poison, nevertheless the experimentation was begun with this phase of the work. Since pharmacologists have determined that, as a rule, nicotine, regardless of how it is administered, has practically the same general effects, it seems logical that nicotine as an insecticide will also have practically the same effect, regardless of how it is applied.

I.—NICOTINE AS A STOMACH POISON

Since the writer, during the past four years, has made a special study of the behavior of the honeybee, and as the honeybee is one of the highest forms of insects, it was first selected for making a special study of the physiological effects of nicotine on this class of animals.

(*a*) BEES FED PURE NICOTINE

To avoid the complications which often arise when a drug composed of more than one constituent is administered, pure nicotine was fed to bees in the following manner: Honey and pure nicotine were thoroughly mixed in the proportion of 1 part of nicotine to 100 parts of honey. Ten c. c. of this mixture were poured into a small tin feeder covered with parallel pieces of wire; then the feeder and contents were placed inside a triangular observation case, previously described by the writer (17). Fifty bees (guards) were next introduced into this case. On account of the faint nicotine odor emitted from the mixture of honey and nicotine, the bees did not eat the food readily. To be certain that the bees had eaten some of this poisoned food before they died, the honey stomachs of several dead bees were examined. It was found that each stomach contained more or less honey, and this was certainly not eaten before the bees were put into the case, because the honey stomachs of guards never contain honey. Testing this supposedly poisoned honey for the presence of nicotine by using alkaloidal reagents, such as silicotungstic acid and phosphomolybdic acid without first attempting to isolate the alkaloid means nothing, for these reagents also precipitate honey and many other

organic substances. This test did not appear sufficiently significant to warrant the expenditure of more time.

The 50 bees lived from 10 hours to 72 hours, with 33 hours as an average, whereas 50 bees fed honey containing no nicotine lived 8 days, on an average. To facilitate description, the behavior of the bees dying of nicotine poisoning may be divided into three stages, and since nicotine kills the higher animals by paralysis and since, as will be shown, it kills insects similarly, the words "paralyze" and "paresize" may be used from the outset. The word "paresis" means partial motor paralysis, while the word "paralysis" includes both motor and sensory paralysis.

FIRST STAGE.—Shortly after being poisoned, bees become more or less inactive and are seldom seen eating. They "pay little or no attention" to hive mates or to strange bees and never attack the latter. They soon become stupid, and from then on their behavior is quite abnormal. All their senses are perhaps benumbed, for they do not offer to attack bees carrying foreign hive odors, and they are not very sensitive to mechanical stimuli of any kind. A little later one or both, but usually one, of the hind legs becomes partially paralyzed (paresized), and thereafter they are of little use. Or the front legs may be stricken partially or totally with motor paralysis at the same time, but occasionally the middle legs may be similarly affected before either of the other two pairs is stricken. The wings seem to be paresized before the legs are affected, because a stupid bee removed from the case is able to walk normally and can vibrate its wings, but can not lift itself from the table. Whenever the motor paralysis has not extended further than to paralyze partially the wings and to paralyze totally only one pair of legs, the bees in almost every instance recover when removed from the case to fresh air and when given pure honey. They eat the honey readily and soon throw off their stupor, and the paresized wings and legs soon recover so that after half an hour the bees are again able to fly.

SECOND STAGE.—Soon after one pair of legs is stricken, all three pairs and the wings become paresized. During this stage bees act somewhat like a man intoxicated with alcohol. They walk in a staggering manner, drag the paresized legs, and frequently fall down, but never walk upright in the normal manner. Sometimes all three legs on one side may be affected totally by motor paralysis, while on the other side one or more legs may not be stricken. In such a case as this, the bee lies flat on its thorax and abdomen and turns in a circle. Often a bee falls over on its side or on its back and can not get up. Sometimes the middle and hind legs are totally stricken with motor paralysis, while the front legs are apparently not affected. In this case the bee crawls along by dragging its abdomen. A little later when all the legs and wings are affected totally by motor paralysis, the bees are entirely helpless. If removed from the case at this instant, a bee thus paralyzed is still able to extend its tongue and to eat honey, but a few moments later the mentum

becomes paresized, and the tongue can no longer be extended. Subsequently the mandibles and antennæ are stricken.

THIRD STAGE.—At the beginning of this stage the bees are apparently dead, except that an occasional twitching of a tarsus or a slight movement of the end of an antenna or of the abdomen may be seen. Sometimes an abdomen passes through a series of convulsions, and occasionally a small amount of feces is voided; in one instance a small amount of liquid was seen issuing from the mouth.

If the behavior of bees dying of nicotine poisoning is interpreted in the same way as is interpreted the behavior of higher animals likewise poisoned, it seems that nicotine as a stomach poison really kills bees by motor paralysis, and that the paralysis travels along the ventral nerve cord from the abdomen to the head, first affecting the abdominal and thoracic ganglia, then the subesophageal ganglion, and last the brain. Considerable light is thrown on this point in the portion of this paper dealing with the tracing of the nicotine from the time it is applied to the time it reaches the nervous system. Having decided that nicotine kills insects by paralysis, we shall now consider the effects of nicotine as an insecticide when applied in practical work.

2.—NICOTINE SPRAY SOLUTIONS

The following results are not meant to test the efficiency of any of the commercial nicotine spray materials, or of even various dilutions of them, but merely to determine how nicotine affects insects when it is applied as in practice.

(*a*) APHIDS DIPPED INTO SOLUTION OF PURE NICOTINE

Carolina poplar leaves bearing many aphids (*Aphis populifoliae* Davis) were dipped into a solution of pure nicotine (1 : 100). At once the aphids began to exhibit an abnormal behavior and soon showed signs of dying. Half an hour later all of them were dead.

(*b*) APHIDS SPRAYED WITH SOLUTION OF PURE NICOTINE

Many more aphids on Carolina poplar leaves were sprayed heavily with the above solution. Half an hour later nearly all the aphids were dead, every one being dead 15 minutes still later. Before being sprayed, these aphids were quiet and seldom moved from place to place on the leaves. They usually stood on the first two pairs of legs, with the hind pair of legs and abdomen high in the air and with the beaks stuck into the leaves. Occasionally an aphid elevated its abdomen higher into the air and simultaneously moved its body sidewise in a jerky manner. The legs and antennæ were moved little, and no liquid was seen issuing from the cornicles or from the anal openings. Immediately after being sprayed, these same aphids lay flat on the leaves, apparently dead.

They were covered more or less with white "wool," which was less conspicuous after being wetted by the spray. Five minutes later the aphids stood up, began to move their legs, and most of them were comparatively active for a few moments. They removed their beaks from the leaves, moved about considerably by lifting the legs nervously, and their peculiar jerky movements became more conspicuous. Later they were more quiet and the legs became paresized, the hind legs being affected first, the middle legs next, and the front legs and antennæ last. At this stage the hind legs generally are totally stricken with motor paralysis. When paresized, many of the aphids fell from the leaves, and for a few moments they seemed to be recovering from their stupor; but they finally died. However, all of those that fell from the leaves lived several moments longer than those that remained on the leaves. When almost inactive, the aphids fell either over on their sides or on their backs and were completely helpless. The last signs of life were twitchings of the tarsi and slight movements of the antennæ. Before death, the bodies of the aphids appeared perfectly dry. When dead, the legs are usually folded and are stiff. During the various stages of paralysis, it was common to see small drops of clear and dark fluids issuing from the cornicles and anal openings.

(*c*) INSECTS SPRAYED WITH SOLUTION OF NICOTINE SULPHATE

Many aphids on leaves of the Carolina poplar (*Populus deltoides*) were heavily sprayed with a solution of nicotine sulphate, made in the proportion of 1 ounce of the nicotine sulphate to ½ gallon of water, this being 1 part of the insecticide to 64 parts of water, which is 12½ times as strong as recommended for the more resistant sucking insects, such as the black aphis and woolly aphis. This nicotine sulphate is guaranteed to be at least 40 per cent nicotine, and the analysis of this sample showed that it contained a fraction more than 40 per cent. Four hours after being sprayed, all these aphids were apparently dead.

In practical work nicotine as an insecticide is rarely used for caterpillars and probably never in the form of spray for the imagoes of coleopterous and hymenopterous insects, but it was desirable to ascertain how nicotine affects various kinds of insects and to obtain material for the study of the tissues after the insects had died of nicotine poisoning. For this reason various kinds of insects were heavily sprayed with the above solution. An hour elapsed before the large caterpillars of the catalpa sphinx (*Ceratomia catalpae* Bdv.) died; however, a much weaker solution (1 : 1,200) apparently killed the small caterpillars (6 to 10 mm. in length) of the same moth in five minutes, and the same was true of an extract made of powdered tobacco and water (50 gm. of tobacco boiled in 1,000 c. c. of water). The stronger solution of the nicotine sulphate quickly killed the small caterpillars of *Atteva aurea* Fitch, and of *Datana* sp., but it was not so effective on the larger larvæ of the lesser wax moth

(*Achroia grisella* Fab.) and on bagworms, larvæ of *Thyridopteryx ephemeraeformis* Haw. Of the four adult blister beetles (*Epicauta pennsylvanica* DeG.) sprayed, only three died, and worker bees could not be killed by spraying them.

From the foregoing it is thus seen that there is considerable difference in the responses of various insects to nicotine spray solutions. The youngest and smallest individuals of any given species always succumb first and some imagoes, such as bees, can not be killed at all.

3.—NICOTINE AS A FUMIGANT.

The following apparatus was devised: To the neck of a 50 c.c. retort supported on a ring stand was connected a piece of rubber tubing 12 inches in length, with its free end projecting into a battery jar 9 inches in diameter and 12 inches in height. The jar was covered with a piece of glass.

(*a*) APHIDS FUMIGATED WITH PURE NICOTINE

Carolina poplar leaves bearing many aphids (*Aphis populifoliae*) were supported in a bottle, and the bottle with its contents was placed inside the battery jar so that the aphids did not touch the sides of the jar. Twenty-five c. c. of pure nicotine were poured into the retort, which was then heated gently. The free end of the tubing was removed from the battery jar, and the heat was still applied. Brownish fumes soon arose from the nicotine; they immediately condensed upon striking the upper, colder portions of the retort, which soon became too warm to condense them. Other fumes then passed into the neck of the retort, where they were likewise immediately changed into liquid, which ran in little streams back into the retort. The rubber tubing was next warmed by the fumes. As soon as drops of the liquid ceased to fall from the free end of the tubing the fumes were passing freely from this end. The tubing was then inserted into the battery jar. At once the aphids began to squirm, and the jar was soon filled with dense fumes. At this instant the burner was removed from under the retort, whereupon the fumes began to condense. A little later small streams of the liquid ran down the sides of the jar, and small drops collected on the underside of the glass cover. The leaves and the aphids seemed to be covered with a fine spray.

So far as could be observed through the dense fumes, the behavior of these aphids was similar to that of sprayed aphids. Before dying many of them dropped from the leaves. Most of them appeared dead within three minutes after the introduction of the fumes; two minutes later still all of them were dead.

The preceding mode of procedure has been described in detail in order to make evident the ease with which the liquid can be applied by fumigating to cool surfaces with which the vapors may come in contact. Since the temperature of insects is practically the same as that of the

air surrounding them, it seems evident that the nicotine fumes would be condensed upon striking the integuments and tracheal walls of the insects fumigated.

The preceding experiment was repeated by using aphids (*Aphis rumicis* L.) on nasturtiums. Small pots containing these plants were placed inside the battery jar. Five minutes after introducing the fumes all the aphids were dead.

(*b*) INSECTS FUMIGATED WITH 40 PER CENT NICOTINE-SULPHATE SOLUTION

The preceding experiments were repeated by fumigating the following insects with a 40 per cent solution of nicotine sulphate: Aphids on nasturtiums (*Tropaeolum majus*) and those (*Myzus persicæ* Sulz.) on potato plants (*Solanum tuberosum*), coccids (*Orthezia insignis* Dougl.), fall webworms (caterpillars of *Hyphantria cunea* Dru.), larvæ of potato beetles (*Leptinotarsa decemlineata* Say), imago house flies (*Musca domestica* L.) and worker honeybees (*Apis mellifica* L.).

The aphids and coccids died a few minutes after the introduction of the fumes, and the plants which bore them were also affected considerably by the fumes. The leaves on the potato plants soon wilted, and some of them finally turned brown. They emitted a comparatively strong nicotine odor for several days, and even a very faint nicotine odor was perceptible 15 days after the plants were fumigated.

The fall webworms and potato-beetle larvæ (two-thirds grown) were not so easily killed, although after being confined for a period of 15 or 20 minutes in dense fumes, they die. While dying, the caterpillars wriggle about considerably and exude a yellowish fluid from the mouths. Bees die in the same length of time, but house flies do not succumb so readily. Bees, when apparently dead, often revive if they are removed from the jar to fresh air.

The preceding experiments indicate that nicotine as a fumigant kills insects by paralysis and that part, if not all, of the fumes, which strike the integuments and which pass into the tracheæ of the insects, are condensed before they enter the various tissues. On page 110 it is shown that the nicotine never passed far from the tracheæ into the tissues. This supports the view that nearly all of the fumes in the tracheæ were changed into liquid which did not pass readily through the tracheal walls. It is also seen that the most delicate insects yield first to nicotine fumes.

4.—NICOTINE ODOR AND VAPOR

To determine the effects of nicotine odor on insects, leaves were either sprayed with or dipped into nicotine spray solutions. Their stems were then inserted into bottles of water which were placed in the sun or in front of an electric fan. The leaves were always left in the current of

air from the electric fan for an hour, at the end of which time they were perfectly dry, but still emitted a very faint odor of nicotine. A slightly longer time in the sun was required before they became perfectly dry. Each bottle with its contents was placed inside a battery jar 5 inches in diameter by 11 inches in height. Normal and untreated insects were then removed with a camel's-hair brush from other leaves to the leaves in these bottles. A glass cover was placed over each bottle, and the insects were observed at regular intervals.

To ascertain the effects of nicotine vapor on aphids and bees, the insects were either inclosed in a battery jar with nicotine spray material below them or with the spray solutions placed in watch glasses or on the leaves near the insects in the open.

(*a*) ODOR FROM SOLUTION OF PURE NICOTINE

Carolina poplar leaves were dipped into a solution of pure nicotine (1:100), placed in the current of an electric fan for an hour, and were then arranged as already described. At 11 o'clock aphids (*Aphis populifoliae*) from other leaves of the same tree were transferred to the leaves treated with the nicotine solution. At 4.30 o'clock the aphids were slightly stupid. The next morning all of them were dead. Not one of the aphids used as controls died.

One day aphids were killed by being placed in vials which a week before had contained some of the nicotine solution. These vials after having been used had not been washed, and a week later two of them were unintentionally used for collecting aphids in the greenhouse. By the time a dozen aphids had been put into each vial and closed with stoppers which had also been used a week before, most of the insects were dead, and the remainder of them were in the last stage of paralysis. An examination showed that the vials still gave off a very slight odor of nicotine.

(*b*) VAPOR FROM PURE NICOTINE

At 10.30 o'clock a large Carolina poplar leaf bearing many aphids was put into one of the battery jars. A small beaker containing 5 c. c. of pure nicotine was also placed inside the jar about 5 inches below the leaf. At 12 o'clock a few aphids were stupid; at 1 o'clock several were dead; at 4.30 most of them were dead; the next morning all of them were dead.

A few cubic centimeters of a pure nicotine solution (1 : 100) were poured into each of seven watch glasses. A small wire screen was laid on top of each watch glass so that it did not touch the nicotine solution. Several cabbage aphids (*Aphis brassicae* L.) were then placed on each wire screen. The smallest aphids died within 10 minutes, the medium-sized ones within 16 minutes, and the largest ones within 22 minutes.

At 11 o'clock the upper surface of a large dock leaf (*Rumex* sp.) was sprayed heavily with the nicotine solution (1 : 100) in the greenhouse. A comparatively large nasturtium leaf was placed directly over and one-half inch above the dock leaf. The under surface of each leaf bore many aphids. At 2 o'clock all the aphids on the underside of the nasturtium leaf were dead, while none on the dock leaf apparently had been affected.

(*c*) ODOR FROM EXTRACT OF POWDERED TOBACCO

Catalpa leaves were sprayed heavily with the extract of powdered tobacco described on page 93. After these leaves had become perfectly dry in the sun, they still emitted a faint nicotine odor. At 1 o'clock many small caterpillars (6 to 10 mm. in length) of the catalpa sphinx were then tested by being placed inside battery jars in the manner already described. At 1.10 o'clock a few of them were dying; at 2.25 four-fifths of them were dead. These had not eaten of the leaves. The remaining ones did not die until three days later; they had eaten the leaves to a limited degree.

The preceding experiment was repeated twice by using large fall webworms on mulberry leaves (*Morus* sp.) that had been submerged for two minutes in the extract and had been dried in the current of air from the electric fan. The first lot of caterpillars ate the leaves readily and apparently were not affected, but a third of the second lot was dead the following morning, after having slightly eaten the leaves.

(*d*) ODOR FROM SOLUTION OF NICOTINE SULPHATE

The experiment just preceding was repeated twice by using a solution of nicotine sulphate (1 : 1,200). The results obtained with the first lot of fall webworms showed that only one caterpillar was killed, but the leaves were not much eaten. Relative to the second lot, the leaves were not eaten at all. Two hours after being placed on the leaves, many of the caterpillars became stupid and a few showed signs of dying, but were not found dead until the following morning. Two days later still all of them were dead.

The preceding was repeated by heavily spraying catalpa leaves with a much stronger solution of nicotine sulphate (1 : 64) and by using small caterpillars (6 to 10 mm. in length) of the catalpa sphinx. At 12.52 o'clock these leaves had become perfectly dry in the sun; at 12.56 a few of the caterpillars acted as if dying; at 2.25 all of them were dead. The leaves gave off a slight nicotine odor and had not been eaten.

The preceding was repeated by dipping Carolina poplar leaves into the same solution and by using the aphids removed from these leaves after the latter had been dried in the current of air from the electric fan. The following morning most of these aphids were dead.

(e) VAPOR FROM A 40 PER CENT NICOTINE-SULPHATE SOLUTION

Fifty worker bees in an observation case were introduced into a large battery jar. A small quantity of a 40 per cent nicotine sulphate solution in a Petri dish 10 cm. in diameter was placed inside the jar 8 inches beneath the case of bees. During all the following day the bees remained more or less inactive and appeared slightly stupid. The next day following they were still slightly abnormal in behavior, but none died.

The upper surfaces of large dock leaves were heavily sprayed with two solutions of nicotine sulphate (1 : 100 and 1 : 500) in the greenhouse. The aphids on the under surfaces of these leaves apparently were not affected by the vapor from either solution. On the other hand, when a small amount of the stronger solution was placed on the under surfaces of nasturtium leaves near the aphids but not against them, most of the insects were found dead three hours afterwards. When a piece of cheesecloth wet with the weaker solution was placed an inch beneath the branches and leaves of a nasturtium, a few of the many insects on this plant were found dead.

In view of the results of all the preceding experiments in which the spray solutions had been evaporated, it may be argued that many of the insects died of nicotine poisoning by eating the leaves which had previously been treated with nicotine solutions. That these leaves still emitted a faint odor of nicotine indicates that their surfaces still bore many traces of the alkaloid. It is also probable that some of the nicotine passed into the tissues of the leaves. Since some of those insects that did eat the leaves died so quickly after being placed inside the jars, it does not seem logical that they died primarily from the effects of nicotine as a stomach poison, because a small amount of nicotine as a stomach poison acts slowly. In view of the preceding reasoning and since some of the insects did not eat the leaves at all, it seems safe to say that most of them were killed by the odoriferous particles of the nicotine passing into the tracheæ. In all of those experiments in which the insects were subjected to nicotine vapor, although they did not actually touch the nicotine solutions, there can be no doubt that the vapors killed the insects; and it is also probable that the vapors passed into the tracheæ and killed by paralyzing the nervous system. These experiments demonstrate that nicotine spray solutions are not necessarily contact insecticides, although they are more effective when actually used as such, for by this means the insects are constantly brought near the vapor under the most favorable conditions.

TRACING NICOTINE TO TISSUES

Owing to the small sizes of the insects used, it was not considered possible to operate successfully on live individuals in order to determine what particular tissue is vitally affected when nicotine is used as an insecticide. Drawing conclusions solely from the behavior of the insects dying of nicotine poisoning, the author states in the preceding pages that they die of

paralysis. Since paralysis is an affection of the nervous system, it still remains to be shown that nicotine applied as an insecticide reaches the nervous system and how it affects the nerve cells. The following pages deal with this portion of the work. Many difficulties were encountered, and the experiments performed to determine how nicotine affects the nerve cells gave no definite answer to this question. The latter phase of this subject is presented mostly by giving a brief discussion of the various views pertaining to the physical and chemical effects of drugs on cells.

Tracing the nicotine into the various insect tissues was accomplished by precipitating this alkaloid immediately after it had killed the insects and then by carefully studying the microscopical sections made from the insects thus tested. A study of this nature involves considerable technique and many precautions in making sections, because two objects instead of one must be successfully accomplished at the same time. It is an easy matter to obtain good sections of most larvæ and soft-bodied insects under ordinary conditions, but it was found quite difficult to obtain good sections and at the same time not to lose the precipitates held in the tissues while the slides were being run through the various reagents. This is appreciated when we consider the solubility of various substances in the clearing oils, in the alcohols, and in water.

In addition to the difficulties enumerated above, there are still three more to be considered: (1) After a certain period has elapsed following death as a result of having been treated with nicotine, the tissues of the insects were unusually abnormal upon fixation. As soon as life is extinct, and probably a short time before, the cells gradually change from normal to abnormal ones. This was particularly noticeable when small caterpillars were sprayed with solutions of nicotine. A short time after death they turned brown and the tissues were found to be more or less disintegrated. For this reason it was always necessary to fix the insects just before the last signs of life had disappeared in order to avoid mistaking post-mortem changes in the cells for physical ones caused by the nicotine. (2) On the other hand, if nicotine really causes physical changes in the cells of insects, these changes are always masked by the large physical ones caused by the fixing reagents. (3) It is often difficult and sometimes impossible to distinguish the precipitated insecticide inside the tissues from the coagulated constituents of the cells caused by the fixative. Fischer (8) regards the coagulation of these constituents, which really constitutes fixation, as a true precipitation, but of course it is a milder form. The coagulated particles are, nevertheless, frequently as large and sometimes larger than the precipitated ones.

1.—TRACING COLORED LIQUIDS INTO INSECTS

Before determining whether or not nicotine spray solutions as applied under practical conditions reach the tissues by passing through the spiracles, many preliminary experiments were performed to ascertain

whether water and nicotine solutions, applied under the most favorable conditions, are able to enter the spiracles, mouths, and anal openings of various insects. In order to follow these liquids, they were colored with various stains.

(*a*) ABILITY OF COLORED LIQUIDS TO ENTER THE SPIRACLES AND ALIMENTARY CANAL

A small quantity each of water and of pure nicotine solution (1:100) was colored with each of the following aqueous stains: Carmine acid (Grübler's Carminsaur), eosin, gentian violet, Delafield's, Ehrlich's, and pure hematoxylins, methylene blue and safranin. Cabbage aphids were submerged in each of these colored liquids for an hour. After removal from the liquids they were well washed in water, then crushed, and finally mounted on slides in glycerin. The glycerin did not mix with nor scatter the stains. Various parts of the integuments were colored more or less with each stain, but methylene blue seemed to penetrate the integuments the most readily of any of these stains. Each stain seemed to pass into the tracheæ more or less, but the four following ones entered most readily: Carmine acid, gentian violet, methylene blue, and safranin. Of these four, carmine acid proved to be the best. The tracheæ in most of the aphids showed scarcely any of the stain, while those in the remaining ones showed it conspicuously. In these few insects all the larger tracheæ in the abdomen, thorax, and head were stained; and occasionally a stained trachea was traced into a leg. There seemed to be no difference in permeability between the stains dissolved in water and those dissolved in the nicotine solution.

The preceding experiments were repeated by submerging roaches (*Periplaneta americana* L.), croton bugs (*Blattella germanica* L.), house flies, and larvæ of blow flies (*Calliphora vomitoria* L.) for an hour in water and in a pure nicotine solution (1:500), each being colored with carmine acid. The stain was observed in the esophagus and anus of the roaches and croton bugs; in a few of the larger tracheæ and in many of the smaller ones and in the hind gut of the fly larvæ. The house flies were fixed in absolute alcohol, which readily throws down carmine acid.[1] One of the flies that had been submerged in the colored nicotine solution was sectioned, and the sections were placed in xylol alone without being stained, in order that none of the "precipitated" carmine acid might be lost. A study of the sections showed the "precipitated" carmine in several of the larger tracheæ (Pl. 1, fig. A, *pr*). An examination of the other flies showed that the colored liquid had passed into some of the larger tracheæ and into the rectums.

The preceding experiments were repeated by submerging green peach-aphids (*Myzus persicae*) in pure nicotine solution (1:500), colored with

[1] Absolute alcohol does not precipitate carmine acid nor indigo-carmine, but merely throws them out of solution, because they are not soluble in it. For lack of an appropriate term to describe these stains when thrown out of solution the word precipitate in quotation marks may be used.

indigo-carmine (sodium sulphindigotate) for 45 minutes. These insects were fixed in absolute alcohol, which readily throws down indigo-carmine. The resulting "precipitate" is totally insoluble in xylol and absolute alcohol, but its solubility in the other alcohols increases as the water in them increases. For this reason the sections of these aphids were stained in absolute alcohol containing safranin. The blue "precipitate" was common on the outside of the integument, but it was not seen inside the integument anywhere, except occasionally in the larger tracheæ and then usually not far from the spiracles. Plate 1, figure B, represents the "precipitate" (*pr*) observed in two places in the same trachea. This drawing was made from two consecutive sections and shows the most "precipitate" that could be found.

The experiment just preceding was repeated by submerging larvæ of wax moths (*Achroia grisella*) and small nymphs of croton bugs in the above liquid for 30 minutes. A small amount of blue "precipitate" (Pl. 1, fig. C, *pr*) was observed in most of the larger tracheæ of the wax moths and occasionally some (Pl. 1, fig. D, *pr*) in the smaller ones. Each of the sections of the croton bugs, containing parts of the alimentary canal, shows more or less blue "precipitate" inside this tube; but none was observed elsewhere inside the integument.

Thirty worker bees were submerged for 30 minutes each in water and in pure nicotine solution (1:500), each liquid being colored with carmine acid. When removed from the liquids, the bees were thoroughly washed in water. The apparently dead bees were then laid on blotting paper in the sun to become dry and to revive from the effects of the liquids. All 30 bees submerged in the colored water revived and were walking about in from 12 to 18 minutes, with an average of 15 minutes, after being placed in the sun. Only 60 per cent of those submerged in the colored nicotine solution revived, and these never became able to fly as did those submerged in the colored water. The time required for them to recover sufficiently so that they could crawl about varied from 45 minutes to 3¾ hours, with about 2 hours as an average.

An examination of the live bees just described showed that the thin chitin between the segments was often colored red and that when a thorax was crushed, the red liquid usually issued from the mouth. When the bees that had been submerged in the colored water were dissected, the stain was seen in 90 per cent of the honey stomachs, in 64 per cent of the ventriculi, in 50 per cent of the rectums, and in 50 per cent of the anal openings and around the stings. When the bees that had been submerged in the colored nicotine solution were dissected, the stain was observed in 45 per cent of the honey stomachs, in only 6 per cent of the ventriculi, never in the rectums, and in 45 per cent of the anal openings and around the stings. The behavior of the bees when placed into the nicotine solution may be used to explain why such a small amount of the colored solution passed into the alimentary

canal. While the bees placed into the colored water struggle and cling to one another for several moments after being submerged, those placed into the colored nicotine solution struggle little and never cling to one another. They seem to be slightly paresized as soon as put into the solution, and perhaps for this reason alone they swallow little of the liquid. Paresis may also be used to explain why the colored liquid is not forced from the honey stomachs into the ventriculi.

That the colored liquids were never seen in any of the tracheæ of the 60 bees submerged demonstrates that the valves guarding the spiracles closed water-tight at the instant of placing the bees into the liquids. That one-half of the bees submerged in the nicotine solution did not swallow any of it indicates that these valves can not be closed air-tight, because there seems to be no way of explaining why the bees were paresized other than by supposing that vapor from the nicotine solution passed the valves and entered the tracheæ. Of course, the vapor might have entered the insects through the mouths and anal openings, but this view is highly improbable, and the liquid had not penetrated the integuments even at the thinnest places.

To determine whether the red liquid passed through the thin chitinous layer of the honey stomachs, several of these organs which were almost full of the red liquid were removed after both ends of a honey stomach had been securely ligatured with thread. Immediately after a honey stomach had been dipped into water to moisten its walls, it was gently rolled on white paper. No red liquid was seen issuing through its walls; nor was any observed on the paper. The same experiment was repeated by using the ventriculi. In this case red liquid was plainly seen to issue from the walls of each ventriculus, and it made the paper red. Thus, it seems that the red liquid usually seen surrounding the viscera of these bees when cut open had not passed through the walls of the honey stomachs, but through those of the ventriculi and probably to a limited degree through the walls of the small intestines and those of the rectums, although it is shown on page 108 that a nicotine solution containing indigo-carmine does not pass through the walls of the small intestine and rectum.

In all the preceding experiments liquids colored with stains have been used. It is probably true that many stains increase the permeability of their solvents and consequently may also increase the ability of the solvents to pass into small openings, such as the spiracles. For this reason coccids (*Orthezia insignis*) were submerged for 30 minutes in a pure nicotine solution (1 : 100) which had not been colored with a stain. They were then fixed for 15 minutes in a mixture consisting of two parts of absolute alcohol and one part of phosphomolybdic acid. To insure the removal of all the phosphomolybdic acid not united with the nicotine and to insure better fixation, the insects were put into a mixture consisting of two parts of absolute alcohol and one part of

glacial acetic acid. After remaining in this mixture for about three hours, they were placed into absolute alcohol for another hour.

Phosphomolybdic acid is one of the alkaloidal reagents, and it precipitates nicotine even in a dilution of 1 to 40,000. It was prepared according to the directions of Autenrieth (1) with modifications as follows: A sodium-carbonate solution was saturated with pure molybdic acid; one part of crystallized disodium phosphate (Na_2HPO_4—$12H_2O$) to five parts of the acid was added and the mixture evaporated to dryness. The residue was fused and the cold melt was dissolved with absolute alcohol. This mixture was filtered and enough nitric acid added to produce a golden-yellow color. The resulting mixture, called "phosphomolybdic acid," was used full strength when mixed with absolute alcohol to serve both as a fixative and as a precipitant. The precipitate resulting from the union of this mixture and nicotine is neither soluble in water nor in any of the alcohols; but for fear of losing some of the precipitate the sections were stained in safranin dissolved in 95 per cent alcohol.

A study of the sections of the coccids treated as described above showed a brownish yellow precipitate inside many of the tracheæ (Pl. 1, fig. E, *pr*), but it was not seen elsewhere inside the integument. In the sections of coccids used as controls no precipitate was seen anywhere. It is thus seen that nicotine solutions containing no stains are able to pass into the tracheæ of coccids that have been submerged in the solution for 30 minutes.

(*b*) ABILITY OF NICOTINE SPRAY SOLUTIONS TO ENTER SPIRACLES

Aphids (*Aphis brassicae*, *A. rumicis* L., and *Macrosiphum sanborni* Gill.), and coccids (*Orthezia insignis*) were sprayed with a pure nicotine solution (1:500), colored with carmine acid, until they were wet with spray. An hour later they were mounted on slides, as described on page 100, and were examined. The spray had evaporated, leaving the red stain adhering to various parts of the integuments. Nearly all of the tracheæ showed no signs of the stain; but a few seemed to be slightly pink, although this kind of an examination is not entirely reliable.

The preceding experiments were repeated by heavily spraying aphids (*Aphis rumicis*) with a pure nicotine solution (1:500), colored with indigo-carmine. The sections were stained as described on page 101. A thorough study of these slides showed that the colored nicotine solution had not passed through the integuments nor into the tracheæ. At only one place was it found that the "precipitate" had lodged in a spiracle (Pl. 1, fig. 6, *sp*); but it was never observed in the tracheæ (Pl. 1, fig. 7, *tr*), nor elsewhere inside the integuments, although it was commonly seen adhering to the outer surfaces of the integuments (Pl. 1, fig. 7, *int*).

Aphids of the same species as just described were heavily sprayed with a pure nicotine solution (1:100), not colored with any stain. These

aphids were fixed with the mixture of absolute alcohol and phosphomolybdic acid and were further treated as described on page 102. No precipitate was found in the tracheæ nor elsewhere inside the integuments.

Some of the caterpillars of the catalpa sphinx (*Ceratomia catalpae*), of *Atteva aurea*, of *Datana* sp., and larvæ of the lesser wax moth, *Achroia grisella*, that had been sprayed with a solution of nicotine sulphate (1:64) were fixed in Carnoy's fluid. This fixative is a mixture composed of equal parts of absolute alcohol, chloroform, and glacial acetic acid, saturated with mercuric chlorid ($HgCl_2$). Mercuric compounds are among the general alkaloidal reagents and the mercury in mercuric chlorid readily precipitates nicotine. The sections of the above larvæ were stained with Ehrlich's hematoxylin and the crystals of the mercuric chlorid remaining in the tissues after the fixative had been washed out were removed by 95 per cent alcohol containing tincture of iodin. The iodin unites with the mercuric chlorid, forming a compound which readily dissolves in alcohol, but iodin apparently has no effect on the gummy precipitate resulting from the union of nicotine and Carnoy's fluid. For this reason, if the spray solution passed into the tracheæ, the nicotine in it should have been precipitated and should not have been affected by the iodin, and all of it certainly could not have been washed out while the slides were being run through the reagents. At any rate, after the sections had been treated with tincture of iodin, no precipitate of any kind was observed inside the integuments of these larvæ. This indicates that a spray solution of nicotine sulphate does not enter the spiracles nor pass through the integument; but this method is not fully reliable, on account of having to remove the crystals of mercuric chlorid.

Since it has been shown that spray solutions, as applied under practical conditions, do not pass into the tracheæ, a study of the spiracles of the aphids, coccids, and larvæ that have been used in the experiments shows that it is practically impossible for aqueous solutions to enter the spiracles. The mouths of the spiracles of all these insects, except the coccids, are guarded by pseudohairs, which are outgrowths from the chitinous linings of the spiracles and by the rims (Pl. 1, fig. J, *r*) turning inward and sometimes downward. The spiracles (Pl. 1, fig. H, *sp*) of the coccids are practically unprotected, while those of aphids (Pl. 1, fig. B, *sp*) bear a few hairs. The small size of these spiracles seems to be the best reason why aqueous solutions can not readily pass into the tracheæ. The hairs guarding the spiracles of some of these insects vary from short, stout ones, as in the larvæ of *Atteva aurea* (Pl. 1, fig. I, *sp*), in the lesser wax moth (Pl. 1, fig. J, *sp*) and in *Datana* sp. (Pl. 1, fig. K, *sp*) to long, stout ones, as in the caterpillar of the catalpa sphinx (Pl. 1, fig. L, *sp*) and in the larva of the Colorado potato beetle (Pl. 1, fig. M, *sp*). The hairs (Pl. 1, fig. N, *hr*) in a spiracle of the fall

webworm are branched, and they nearly close the entrance, while the entrance of a spiracle (Pl. 1, fig. O, *sp*) in the tomato worm (larva of *Phlegethontius sexta* Joh.) is closed by a hairy and porous plate (Pl. 1, fig. O, *p*), which has an oblong opening through its center.

Shafer (20) colored kerosene with Sudan III; and after thoroughly spraying or dipping grasshoppers (*Melanoplus femoratus* Burm.), tomato worms, and aphids into this oil and after dissecting these insects, he found more or less of the red oil in the larger tracheæ. He colored kerosene emulsion and the emulsions of the miscible oils with indigo-carmine and with safranin and found that they also enter the spiracles. Shafer repeated these experiments by treating aphids with creolin emulsion containing indigo-carmine. After fixing the insects in absolute alcohol and after studying the sections he observed plugs of "precipitated" indigo-carmine in the larger tracheæ, which were sufficiently large to close them.

Dewitz (7), in discussing contact insecticides, does not believe that either liquids or powders can enter the spiracles in sufficiently large quantities to cause the death of the insects by suffocation.

Without attempting to apply the physical law governing the surface tension of liquids, the following experiment was performed to determine roughly the surface tensions of water, different solutions of 40 per cent nicotine sulphate, pure nicotine, kerosene, gasoline, and kerosene emulsion. Fresh nasturtium leaves were spread out flat on a table, with the under surfaces upward. With pipettes drops of water, solutions of nicotine sulphate (1:500 and 1:100), undiluted 40 per cent nicotine sulphate, undiluted pure nicotine, pure nicotine solutions (1:500 containing indigo-carmine and 1:100), kerosene, gasoline, and kerosene emulsion were dropped upon the nasturtium leaves. Of all these liquids the surface tension of gasoline was weakest and that of water the strongest; that of kerosene was second weakest, while those of pure nicotine and kerosene emulsion were about equal, but still much stronger than that of kerosene. So far as practical work is concerned, the ability of 40 per cent nicotine sulphate and its solutions and of the two enumerated solutions of pure nicotine to spread over the surfaces of these leaves is about equal to that of water. The drops of each one of these liquids upon striking the leaves form small spheres and are not retained when the leaves are somewhat inclined.

In regard to the evaporation of the solutions of nicotine sulphate and of pure nicotine, the drops of the solutions of pure nicotine evaporated rather quickly while those of the nicotine sulphate did not disappear for some time. The more nicotine contained in the drops of the pure nicotine solutions, the more quickly they evaporated. The evaporation of the drops of the solution of nicotine sulphate (1:500) was about equal to that of the water drops.

From what we know about the relative surface tensions of nicotine solutions, of kerosene, and of various emulsions, it is easily understood why kerosene and the emulsions are able to pass into the tracheæ while the nicotine solutions can not.

2.—TRACING NICOTINE AS A STOMACH POISON TO TISSUES

To obtain material for tracing nicotine as a stomach poison to the tissues and to determine the effects of an extremely small amount of nicotine and of indigo-carmine on bees, the following experiments were performed: 200 drops of pure honey were put into a feeder; 200 drops of honey mixed thoroughly with 40 drops of water colored blue with indigo-carmine were poured into a second feeder; 200 drops of honey mixed thoroughly with 40 drops of pure nicotine solution (1:500) colored blue with indigo-carmine were poured into a third feeder. Each of these feeders, with its contents, was placed inside an observation case, and 50 worker bees were introduced into each case. Before all the bees died nearly all the food had been eaten. Since bees confined in observation cases can not void their feces, the abdomens of these bees became much distended with the blue-green food. The bees that ate the pure honey lived eight days, on an average, while those in the two other cases lived about seven days, on an average, showing that the extremely small amount of nicotine did not affect their longevity, whereas the indigo-carmine seemed to shorten their lives by one day.

The preceding experiments in feeding bees nicotine and indigo-carmine were repeated; and three days later, when several bees showed signs of dying, they were placed into absolute alcohol for two days. The anterior portions of their abdomens, and occasionally the base of a leg, appeared blue-green from the outside. When cut open under absolute alcohol, all the tissues in the abdomen appeared blue-green. A closer examination, however, showed that the alimentary canal was blue, while the other tissues in the abdomen as a rule were pale blue-green, with now and then darker colored streaks running through them. A few muscle fibers and some parts of the chitin were pale blue, and other parts of the chitin were pale blue-green. Under alcohol the tissues in the thorax and head did not appear colored at all; but after being removed from the alcohol and dried, they assumed a pale blue-green color, and occasionally darker colored blue streaks were seen in the muscles and brain. It seems that the indigo-carmine had colored the blood or body fluid pale blue-green and that this fluid in turn had colored all the tissues, but the stain was diluted too much to be "precipitated," except in a few organs.

Parts of the alimentary canal and various tissues were dissected out, and sections were made of them. Sections through the anterior and middle portions of the honey stomach failed to show any blue "precipi-

tate" either in the lumen or in the walls of this organ, but sections through the posterior portion of the honey stomach and other parts of the alimentary canal distal to the honey stomach usually showed more or less blue "precipitate." There was no difference in distribution of the stain, whether or not it contained nicotine, but since the distribution of nicotine only is of interest, the discussion will be confined to the distribution of the stain which formerly contained this insecticide.

Plate 2, figure D, represents the blue "precipitate" as seen in the wall of the lower portion of the honey stomach. The stain seemed to have united with the alcohol as the former was passing through the chitinous layer (*chl*) of this organ. A little "precipitate" was also seen in the muscular layer (*m*) of this organ.

Sections through the anterior portion of the valve of the proventriculus show small particles of "precipitate" in the muscles (Pl. 2, fig. E, *m*), epithelial cells (*ep*), and tracheæ (*tr*).

Sections through the ventriculus show "precipitate" in various places of the epithelium, indicating that the stain was in the act of passing through the wall when it was overtaken by the alcohol. From the location of the blue "precipitate" some of the stain was just ready to pass into the inner ends of the epithelial cells, while some had just entered these cells (Pl. 2, fig. A, *pr*). Other portions of the stain were "precipitated" midway between the inner and outer walls of the epithelium (Pl. 2, fig. B, *pr*), while still other portions were overtaken by the alcohol when they were passing through the outer wall of the epithelium (Pl. 2, fig. C, *pr*). At this location a small amount of "precipitate" was also seen in the transverse muscular fibers (Pl. 2, fig. C, *tm*), indicating that, while most of the stain passed between the muscular fibers, some of it passed into and probably through the fibers.

At only one place was blue "precipitate" (Pl. 2, fig. H, *pr*) observed in the blood (*bl*). This was seen a short distance from the ventriculus near a small trachea (*tr*) and two Malpighian tubules (*mal*), which also contain a little "precipitate." Two particles of this "precipitate" are lying in the outer walls of these tubules, indicating that the stain was passing into these organs when it was thrown down. It was also observed that the trachea contained several small particles of "precipitate."

Many of the Malpighian tubules, particularly those near the honey stomach, small intestine, and rectum, showed no traces of the stain, while those near the ventriculus contained a small amount of it, as represented in Plate 2, figure H, and whereas those against the ventriculus contained large amounts of the "precipitate," as represented in Plate 2, figure G.

The "precipitated" particles in sections through the middle of the ventriculus are more numerous and more compact than in sections through either end of this organ. These sections are never perfect,

and since the tissues were fixed with absolute alcohol, which must have passed into the bees chiefly through the mouths and anal openings, the cells in the epithelial lining of the alimentary canal were not well preserved, so they have been drawn diagrammatically in outline from Snodgrass (22). In sections through the middle of the ventriculus, the "precipitate" is arranged more or less in concentric circles (Pl. 2, fig. J, *pr*). This arrangement is probably caused by the peritrophic membranes being likewise arranged. In the lumen (*l*) and between the concentric circles of "precipitate" the "precipitated" particles are scattered irregularly. In Plate 2, figure J, the epithelial (*ep*) and muscular (*m*) walls have been drawn diagrammatically, showing how the stain probably passes through these walls into the blood where most of it is taken up by the Malpighian tubules. The other parts of this figure were drawn with the aid of a camera lucida.

Since no blue "precipitate" was observed in the epithelium (Pl. 2, fig. F, *ep*) of the small intestine, it seems that the stain did not pass through the walls of this organ, although "precipitate" was easily seen in the lumen (*l*) of the small intestine. Most of the "precipitate" usually occurred in large particles near the center of the lumen, while the inner wall of the epithelium was often lined with a layer of "precipitate" composed of innumerable small particles.

Despite the fragmentary sections of the rectum, a careful study was made of this organ, but no blue "precipitate" was seen in its walls; nevertheless it was quite conspicuous in its lumen (Pl. 2, fig. I, *l*). In the rectum, as well as in the other parts of the alimentary canal, there was considerable "precipitated" material which was not stained. This and the pollen grains were easily distinguished from the "precipitated" indigo-carmine by the blue color of the latter.

The preceding results obtained in tracing nicotine as a stomach poison by means of "precipitating" indigo-carmine is not meant to be conclusive, but merely to point out the possibilities for future investigations along this line. For these results to be conclusive, the nicotine should have been traced without the aid of a stain like indigo-carmine; but owing to the odor from this insecticide bees can not be forced to eat food containing a large quantity of nicotine. For this reason it did not seem possible in preliminary work of this nature to be able to trace an extremely small amount of nicotine without using some comparatively harmless stain with it.

In passing through the walls of the ventriculus, it is scarcely possible that the nicotine and indigo-carmine were separated from one another, and the experiments on page 91 show that nicotine as a stomach poison kills by paralysis. It must therefore be concluded that nicotine in passing through the walls of the ventriculus is not so materially changed as to destroy its effectiveness. That all the tissues, even including the brain, were stained more or less with the indigo-carmine

shows that this substance was widely distributed, and it is also logical to think of the nicotine accompanying the stain wherever it went, except when the stain penetrated hard tissues, such as chitin. In the higher animals nicotine is chiefly excreted through the kidneys, because it is found in the urine soon after it has been administered. Since the Malpighian tubules take up indigo-carmine so readily, it seems that these organs would also readily excrete poisons contained in the blood. As indigo-carmine does not seem to pass through the walls of the small intestine and those of the rectum, they are either impermeable to this substance or the stain has been so changed that it has lost its original permeability. The same reasoning might also be used for nicotine or any other stomach poison which acts similarly, although according to Cushny (6) iron behaves quite differently when administered to the higher animals. He says:

> Iron injected into the veins of animals is stored up in the liver, spleen and bone marrow, but is taken up from these organs again, and is excreted by the epithelium of the cæcum and colon. When iron is given by the mouth, therefore, it may either pass along the canal and be thrown out in the fæces, or it may be absorbed, make a stay in the liver, be excreted in the large intestine, and again appear in the stools.

He also states that iron has been followed in its course through the tissues by histological methods, but nothing is known about the changes which iron preparations undergo in the stomach and intestine, or the form in which iron is absorbed.

3.—TRACING NICOTINE AS A FUMIGANT TO NERVOUS SYSTEM

While experimenting to determine the physiological effects of nicotine as a fumigant, various insects were fumigated with pure nicotine and a 40 per cent nicotine-sulphate solution. The results indicated that the nicotine fumes were condensed wherever they went. Several of the green peach aphids (*Myzus persicae*) that had been killed by the fumes from the solution of 40 per cent nicotine sulphate were fixed with the mixture consisting of absolute alcohol and phosphomolybdic acid. A careful study of the sections made from these aphids gives the following results:

As already stated, these aphids appeared to be covered with fine spray before they died, indicating that the fumes had changed into tiny drops of liquid. In the sections it is easily seen that the entire integument is covered with minute particles of precipitate. Plate 3, figure B, taken from a molting aphid, well represents the precipitate (*pr*) on the integuments which have been cut obliquely. It is to be noted that the minute precipitated particles lie on the outside of both the old (int_1) and new integuments (*int*), and even between them, but never on the inside of the new integument. Aphids, not fumigated, put into the same fixative occasionally show a little precipitated material on the outside of the integuments, but usually it is easily distinguished from the precipitate

resulting from the union of the fumes of nicotine sulphate and phosphomolybdic acid. It is practically impossible to find an aphid or perhaps any other insect that does not carry at least a little organic matter on the integument. Bees, for instance, when placed into silicotungstic acid or into any other alkaloidal reagent soon become more or less covered with a white precipitate, showing that the hairs are full of organic matter.

It is supposed that some of the nicotine fumes which had passed into the tracheæ had not changed into liquid by the time the insects were fixed, and in order to prove that the fixative precipitates nicotine, whether in a liquid or in a gaseous state, a test tube was filled with the fumes from the nicotine sulphate. Immediately after a small quantity of the fixative was poured into the test tube a yellowish precipitate was thrown down.

Upon examining the sections that had not been stained, a few of them were observed to have a light-tan color and the chitin in places assumed a darker tan color. All the aphids after being fumigated assumed a light-tan color; this color was particularly noticeable when the insects were embedded in white paraffin. The same species of aphids, not fumigated, had a whitish appearance. The light-tan color in most of the sections is caused by minute particles of tan-colored precipitate on the integument, in the tracheæ, and to a limited degree in the tissues, but in a few cases the tan-colored tissues contain no perceptible precipitate. In such instances the fumes must have penetrated the cells and mixed with the protoplasm before the cell constituents were coagulated.

Most of the tracheæ contain more or less tan-colored precipitate, but very little of it lies outside the tracheal walls. Plate 3, figure A, represents a large trachea (*tr*) cut both crosswise and longitudinally near a spiracle, showing the precipitate (*pr*) inside the tracheal walls and some scattered in the fat cells (*fc*) which surround the trachea. Tracheæ may be traced for short distances between the cells of any tissue, but the precipitate is never found further from the tracheæ than that shown in Plate 3, figure A. It is quite probable that some of it which lies in the cells has been dragged there from the tracheæ by the microtome knife.

Baker (2) has well described the respiratory system of the woolly apple aphis (*Eriosoma lanigerum* Hausm.), and he gives two drawings, one representing the dorsal tracheal system and the other the ventral tracheal system. To illustrate how well a gaseous or vaporous form of an insecticide may be distributed to all tissues, these two figures are again given (Pl. 3, fig. D, E). It is to be noted that there are seven pairs of abdominal spiracles and two pairs of thoracic ones and that in the abdomen a short distance from each spiracle the trachea divides into two smaller branches, one of which passes dorsally to help form the dorsal tracheal system (Pl. 3, fig. D) and the other passes ventrally to help form the ventral tracheal system (Pl. 3, fig. E). In the ventral system of the thorax there are two ventral arches, while in the dorsal system of the abdomen there is only one, the dorsal arch (Pl. 3, fig. D, *da*). The anterior ventral arch (Pl. 3,

fig. E, *ava*) in the thorax unites the pair of spiracles in the prothorax and aerates the subesophageal ganglion, whereas the posterior ventral arch (*pva*) connects the pair of spiracles in the metathorax and aerates the large thoracic ganglion. The two ventral arches are of the greatest interest, because they and a few other smaller branches carry nicotine fumes directly to the nervous system, and for this reason it is understandable why the fumes so quickly paralyze aphids.

Plate 3, figure F, is a reproduction of a combination drawing from five consecutive sections through the thorax of an aphid that had been fumigated with a solution of 40 per cent nicotine sulphate, showing the precipitate (*pr*) on the integument (*int*), in the tracheæ (*tr*), and in the subesophageal ganglion (*sg*). The large trachea was cut crosswise near the spiracle, and the branches are drawn in only their approximate positions. It is to be noted that the anterior ventral arch (*ava*) passes over the subesophageal ganglion, but sends one of its branches under and into this ganglion. Another large branch from the main trachea also sends one of its branches to the same ganglion, penetrating its dorsal surface. These sections did not actually show the small tracheæ penetrating this ganglion, but sections from several other aphids did.

Plate 3, figure C, reproduces a combination drawing from six consecutive sections of the same aphid as above described, showing three tracheal branches entering the thoracic ganglion. Attention is to be called to the precipitate (*pr*) in these tracheæ and in the ganglion. Often large granules resembling precipitated particles lie in and near the ganglia. Three groups of them are represented in this figure, two being near the largest trachea and one by the smaller trachea. These fine particles may be either the precipitate resulting from fumes that had passed through the tracheal walls, or that from some other source. There can be no doubt about the large particles of the precipitate, because they are never found in aphids used as controls.

While it is easy to find tracheæ and precipitate in the ganglia, it is quite difficult to find them in the brain. This seems to be due chiefly to the absence of the larger tracheal branches in the brain. Plate 3, figure H, shows a small tracheal branch in the brain cut crosswise, containing three particles of the precipitate, and there are a few more scattered in the adjacent brain. Plate 3, figure G, shows a small tracheal branch running into an optical lobe, containing a few particles of the precipitate.

A critical study of any given tissue would certainly show that it contains as much precipitate as found in the nerve tissue; but no other tissue was thus studied, because all the evidence indicated from the outset that nicotine kills insects by paralysis. One more illustration from the aphid may be used to show that the precipitate may also be found in tracheæ aerating other tissues. Thus, Plate 3, figure I, represents a tracheal branch containing the precipitate running between two ovaries.

Two of the recently emerged house flies that had been fumigated were fixed with the mixture of absolute alcohol and phosphomolybdic acid, and were sectioned. Most of the sections were not cleared well and consequently are not reliable for a study of this kind, but a few of them are fairly reliable. Plate 3, figure M, represents a spiracle and a portion of a trachea taken from the abdomen of a fly. The neck of the spiracle was almost closed with the precipitate (*pr*), while scattered particles of it were seen along the walls of the trachea. Plate 3, figure L, represents two medium-sized tracheæ (*tr*) and a large fat cell (*fc*), taken from another section through the abdomen, showing fine particles of precipitate (*pr*) inside the tracheæ and in the fat cell outside the nucleus.

In conclusion, under this head a few more remarks may be made. A ganglion is composed usually of two more or less round or oblong halves which are securely united to one another. The outer or cortical layer is cellular, while the center of each half never shows definite cell walls or nuclei like those in the cortical layer. There is also usually a difference in coloration between these two portions after being stained, although this difference in the aphids stained with safranin was scarcely noticeable, and the cortical layer was not cellular. This was true not only for those that had been fumigated, but also for the controls that had been fixed and stained the same way. In the illustrations the two portions are distinguished by a difference in stippling. At no time was any anatomical change observed in any insect that could actually be attributed to the effect of nicotine. The failure to see such changes, if they existed, is not significant, because the physical changes effected by the fixation probably mask the smaller physical changes brought about by the nicotine. In the higher animals, however, it has been observed that nicotine causes slight anatomical changes in the cortical layer of the brain.

In cross sections of caterpillars the tracheæ are easily traced into the ganglia. Most of them penetrate the neurilemma and pass between the two halves, where they ramify considerably by sending minute branches through both portions of a ganglion. Occasionally a small tracheal branch may enter a ganglion near or at the base of a nerve. The ramifications of tracheæ inside the ganglia of aphids are not so easily observed, but they seem to exist, although perhaps not so abundantly.

All the preceding histological work has shown that nicotine spray solutions, and even nicotine used as a fumigant, do not penetrate chitin. To determine whether pure nicotine, undiluted, is able to penetrate chitin, larvæ of house flies, of the lesser wax moths, and aphids (*Aphis rumicis*) were submerged in this fluid for 35 minutes. A study of the sections made from these insects showed that the nicotine had passed into the newly-formed chitinous walls of the tracheæ in the larvæ of the house flies and wax moths, but had not passed all the way through them. Plate 3, figures J and K, representing cross sections of small tracheæ, well illus-

trate this point. These tracheal walls certainly were not much harder than the other tissues, because they stained more deeply than did the older chitin. Plate 3, figure O, represents another trachea of the wax moth, showing an older chitinous tracheal wall; the nicotine did not pass into this wall. Plate 3, figure P, represents a small portion of the integument (*int*) of an aphid, showing that the pure nicotine did not pass into the chitin. Plate 3, figures N and Q, illustrates how well Carnoy's fluid penetrates hard chitin. The black dots in the illustrations (*pr*) are crystals of mercuric chlorid that have remained after the fixative was removed. Plate 3, figure N, represents a trachea from a wax-moth larva, and Plate 3, figure Q, a portion of the integument (*int*) and fat cells (*fc*) from an aphid. Attention is called to the mechanical or physical changes brought about in the fat cells by the fixation. Live fat cells never have a netlike appearance, but appear more or less granular, and usually contain many globules. Sections from other insects that had been fixed in Carnoy's fluid showed better than does figure Q the ability of this fluid to penetrate the integument. In a few cases the crystals lie in rows penetrating the integument, indicating that the fluid had passed through the chitin in streams.

If the most important results recorded under this large heading are briefly summarized, the following conclusions may be drawn: (1) Nicotine spray solutions neither enter the spiracles nor pass through the integuments of insects; (2) nicotine as a stomach poison seems to be distributed to all the tissues, including the nervous system; (3) nearly all the nicotine fumes that strike the integuments and pass into the tracheæ are immediately condensed, so that in regard to nicotine as a fumigant the integuments and tracheal walls are more or less covered with fine spray; (4) this fine spray is well distributed through the many small tracheal branches to all the tissues, where some of it passes into the cells; (5) the nervous system receives its quota of the fine spray and vapors from the spray, which immediately paralyzes the nerve cells; (6) the statement just preceding explains how odoriferous particles and vapors from nicotine spray solutions kill insects by paralysis.

HISTORICAL REVIEW

After making a few remarks concerning the chemistry and properties of nicotine, a brief review pertaining to the pharmacological effects of nicotine on various classes of animals, and a few other observations by the writer will be given in order that these results may be compared with those obtained on the insects discussed in the preceding pages.

(1) GENERAL REMARKS ABOUT NICOTINE

Nicotine ($C_{10}H_{14}N_2$) was conclusively prepared synthetically by Pictet and Rotschy (19) in 1904. This investigation concluded a long series of works pertaining to the structure of this deadly poisonous alkaloid. These authors showed that it is a pyridin-methyl-pyrrolidin.

Blyth (3, p. 271–272) says that nicotine—

> When pure, is an oily, colorless fluid, of 1.0111 specific gravity at 15°. It evaporates under 100° in white clouds, and boils at 240°, at which temperature it partly distils over unchanged, and is partly decomposed—a strong resinous product remaining. . . . It has a strong alkaline reaction . . . and a sharp caustic taste. It absorbs water exposed to the air, and dissolves in water in all proportions, partly separating from such solution on the addition of a caustic alkali.

The aqueous solution acts in many respects like ammonia, saturating acids fully; and by the action of light pure nicotine soon becomes yellow, then brown and thick, in which state it leaves, on evaporation, a brown resinous substance.

(2) PHARMACOLOGICAL EFFECTS OF NICOTINE ON VARIOUS CLASSES OF ANIMALS

Greenwood (9) experimented on certain protozoa, cœlenterates, the earthworm, certain echinoderms, crustaceans, and on certain mollusks by using nicotine. He found that the toxic effect of this alkaloid on any organism is determined mainly by the degree of development of the nervous system. Thus, for the protozoa that he used it can not be regarded as exciting or paralyzing, but is rather inimical to continued healthy life. He states (p. 604) that—

> As soon as any structural complexity is reached the action of nicotin is discriminating, and discriminating in such a fashion that the nervous actions which are the expression of automatism—which imply coordination of impulse—are stopped first. This is seen dimly in Hydra, and it is more pronounced among the medusae, where spontaneity, irradiation of impulse and direct motor activity are affected successively.

He asserts that relative to the higher invertebrates the paralyzing action of nicotine is preceded by a phase of stimulation; and as the positively exciting action becomes noticeable, nicotine becomes more and more a medium in which life is impossible. He found that animals closely allied structurally may also often behave quite differently toward nicotine.

The present writer carried on one preliminary experiment to ascertain the action of nicotine on the lower invertebrates. A piece of scum containing many paramecia and nematodes was placed on a slide under a cover glass. A drop of pure nicotine was then placed at the edge of the cover glass and the following results were observed. The nicotine gradually passed under the cover glass by mixing with the water, and as quickly as it came in contact with the nematodes they began to squirm vigorously, while the paramecia apparently were not affected. A little later the nematodes formed themselves into spirals and lay apparently paresized; then suddenly the spirals unfolded. This kind of behavior continued until the nematodes were no longer able to move. By this time it was observed that the nicotine had passed into their bodies, and later the tough cuticles were constricted and contained many

deep grooves. The paramecia were still alive when the nematodes became lifeless, but they finally died slowly and gradually and at no time showed any reaction which could be attributed to a stimulation.

There are many papers dealing with the economic importance of nicotine as an insecticide, but they contain nothing about the pharmacological effects of nicotine and little about its physiological effects, except that it is effective.

So far as known to the writer, only two authors have anything to say about the pharmacological effects of nicotine on insects.

Del Guercio (10) sprayed silkworms with various dilutions of nicotine and determined that this insecticide within a short time brings about convulsive movements in the caterpillars, causing them to fall from the plants and resulting in death in most cases. He thinks that nicotine spray solutions affect insects by means of the vapors from the nicotine poisoning them and that these vapors even in minute quantities cause irritation and convulsive movements which result in death by total paralysis. He made no histological study to ascertain what tissue is vitally affected, and his view is based solely on the behavior of the caterpillars treated.

Shafer (20) ascertained that insects subjected to the vapors of nicotine and other contact insecticides first pass through a stage of excitement, then through a stage of depression in which the coordination of movements is uncertain, and finally through a stage in which there is total loss of movement and sensibility. The last stage was followed more or less rapidly by death. During the first stage the action of the heart was increased and was irregular, then it became depressed, but the heart action was one of the last visible signs of life to disappear. Secretions were also observed to issue from the mouths. The value of the respiratory ratio arose, showing that these vapors depress the activity of oxygen absorption more than they do the ability of carbon-dioxid excretion. Shafer found that the insects used continued to give off carbon dioxid when no oxygen was present to be taken up. Loeb (16) cites similar experiments in which muscles deprived of oxygen continued to give off carbon dioxid.

Before discussing the pharmacological effects of nicotine on the vertebrates, the physiological classification of this alkaloid as defined by toxicologists may be given. Blyth (3, p. 269–279) places nicotine in that class of poisons affecting the nervous system which causes convulsive movements and complex nervous phenomena. Kobert (12) places nicotine in that class of poisons affecting the cerebrospinal system which is able to kill without producing coarse anatomical changes. Brundage (4) classifies nicotine as a neurotic which depresses the cerebrospinal system.

Blyth (3) says that small fish die within a few minutes from a milligram of nicotine. They are first stimulated, then become less active, and are rapidly paralyzed.

The successive stages of nicotine poisoning in the frog are briefly summarized by Langley and Dickinson (14) as follows: (1) Stage of excitation; (2) stage of spasms; (3) stage of quiescence; (4) stage of flaccidity; (5) stage of paralysis of the central nervous system; (6) stage of paralysis of the motor-nerve endings.

Blyth (3, p. 273) says, "Birds also show tetanic convulsions, followed by paralysis and speedy death," and Sollmann (23, p. 262) asserts that—

> Nicotine is one of the most fatal and rapid of poisons; the vapor arising from a glass rod moistened with it and brought near the beak of a small bird causes it to drop dead at once, and two drops placed on the gums of a dog may cause a similar result.

According to Langley and Dickinson (14), the symptoms of nicotine poisoning in rabbits, cats, and dogs are in a general way similar, and may be briefly described as follows: There is a preliminary excitement; clonic spasms; twitchings of the muscles in various parts of the body; stimulation of the central nervous system; paralysis of the motor-nerve endings in the skeletal muscles; quickening and deepening of the respiration, followed by slowing and cessation; dilation of the pupils; paralysis of the cervical sympathetic system; rise and fall of the blood pressure; rise of temperature; constriction of intestines, followed by dilation, and slight vomiting in cats and dogs. If the doses are sufficiently large, the cerebrospinal system is totally paralyzed.

According to Blyth (3), the symptoms witnessed in mammals poisoned by nicotine are quite similar. With large doses, there is a cry, one or two shuddering convulsions, and death; with smaller doses, there is trembling of the limbs, excretion of feces and urine, stupor, a staggering gait, and then the animal falls on one side. One or two drops of pure nicotine may kill a rabbit, cat, or dog within five minutes. Vas (24) found that the substance resulting after washing tobacco smoke affects the health of rabbits; they lose weight, the number of blood corpuscles is decreased, and the hemoglobin of the blood is diminished. According to Blyth, nicotine also affects horses similarly to the smaller domestic animals.

Blyth says that Dragendorff ascertained that nicotine is absorbed into the blood and is excreted unchanged, in part by the kidneys and in part by the salivary glands.

Krocker (13) was among the first investigators to determine the pharmacological effects of nicotine on man. He found that it paralyzes the nervous system and that death is caused by the rapid benumbing and paralysis of the respiratory center, but not from heart paralysis, although nicotine powerfully influences the action of the heart.

Holland (11) states that two or three drops of the alkaloid is fatally poisonous to man when taken into the stomach, and that death is caused by heart failure. In this latter statement other authorities do not agree with him, for they say that death is due to asphyxia, on account of the paralysis of the respiratory center. In the lower vertebrates the heart still beats some time after life is extinct.

Sollmann (23) summarizes the symptoms of nicotine poisoning on man as follows: The whole cerebrospinal axis is first stimulated, then depressed from above downward; symptoms from large doses resemble those of asphyxia or hydrocyanic acid. Action on the medullary centers is marked and violent; respiration is at first increased, then markedly depressed; paralysis of the respiratory center is the cause of death. Action on the spinal cord consists in strong stimulation of the motor cells, producing convulsions, passage of feces and urine. Nicotine acts on unstriped muscles, paralyzing the ganglia after a brief stimulation. There is nausea and vomiting, violent peristalsis, and even tetanic contraction of the intestine and diarrhea. The respiration, heart strength, and blood pressure are increased; the heart rate is decreased. A strong nicotine solution applied directly paralyzes the nerve fibers. "Free nicotine is caustic on account of its alkalinity." The fatal dose for man is about 60 mgm.; one cigar contains enough nicotine to kill two persons, if it were directly injected into the circulation. "It acts with a swiftness only equaled by hydrocyanic acid."

Cushny (6, p. 304–314) asserts that poisonous doses of nicotine administered to man or other mammals cause a hot, burning sensation in the mouth which spreads down the esophagus to the stomach and is followed by salivation, nausea, vomiting, and sometimes purging. Mental confusion, muscular weakness, giddiness, and restlessness are followed by loss of coördination and partial or complete unconsciousness. Clonic convulsions set in later and eventually a tetanic spasm closes the scene by arresting the respiration.

Autenrieth (1, p. 87) says that nicotine is absorbed from the tongue, eye, and rectum within a few seconds, but from the stomach somewhat more slowly. Its absorption is also possible from the outer skin, and it is eliminated through the lungs and kidneys.

In concentrated form nicotine is a local irritant, though owing to the rapidity of its toxic action, it does not behave like a true corrosive nor does it cause inflammation of the mucous lining of the stomach after a lethal dose.

(3) PHYSICAL AND CHEMICAL EFFECTS ON THE CELLS

In the preceding discussion it has been pointed out that nicotine paralyzes the respiratory center in the brain of vertebrates, causing death by asphyxiation. This implies that, while the nervous system is benumbed and rendered inactive, the lungs are prevented from functioning, and consequently the cells in the tissue die for want of oxygen. Since the invertebrates are differently organized, particularly in regard to their respiratory system, an investigation will be made as to the period insects can live without free oxygen.

Walling (25) states that grasshoppers confined in pure carbon dioxid for 15 hours recover, and Shafer (20) determined that beetles (*Passalus*

cornutus Fab.) confined for 24 hours in pure hydrogen completely recover when placed in fresh air. These experiments indicate that nicotine does not kill insects merely on account of the paralysis of the respiratory centers, because the tracheæ and tissues of an insect contain enough oxygen to keep the cells alive for several hours. Since the cells of insects are constantly surrounded by air containing oxygen, an investigation will be made as to whether or not nicotine interferes with oxidation in the cells and whether it kills physically or chemically.

Greenwood (9) observed that when simple animals die from the effects of nicotine, death is often associated with injury to the cell contents so that they tend to disintegrate. This is shown in the protozoan, *Actinosphaerium* sp., and in the coelenterates, *Hydra* spp. and *Medusa* spp.

Budgett (5) treated infusorians with a number of poisons, including nicotine, and found that these protozoa become strongly vacuolated and finally the membranes burst, allowing the protoplasm to flow out into the water. The same structural changes occurred when he deprived them of oxygen. He says (p. 214): "This indicates that either these poisons prevent oxidation or that lack of oxygen produces toxic substances." He also believes that these poisons not only reduce the normal resistance to the entrance of water but lead to the taking up of water, probably by hastening the molecular breakdown and so increasing the osmotic pressure within the cell.

Loeb (16) and others have experimented extensively with amebæ and paramecia by depriving them of oxygen. They always observed the same structural changes as already cited from Budgett. Loeb also performed many experiments by depriving the eggs of a certain fish of oxygen. He exposed the eggs to a current of hydrogen and observed—

> The liquefaction of the cell walls and the formation of droplets began when the egg was in the 8-cell stage (Fig. 2). These droplets fuse into larger drops and finally nothing but these drops indicates the existence of the germinal disk.

The present writer placed living fat cells and œnocytes of the honeybee on a slide in water under a cover glass. These cells live in tap water for some time before any changes in their appearance can be observed; but when a drop of pure nicotine is placed at the edge of the cover glass, changes in their general appearance take place soon afterwards. The globules in the cells sometimes dance about, resembling the Brownian movement. The globules in the fat cells usually soon lose their rotundity, become massed together, and form a coarse, granular structure. The refractive bodies in the œnocytes soon disappear, and then these cells become opaque. After considerable time the cell walls of the fat cells and œnocytes burst, and the cell contents disintegrate.

In the preceding pages it is shown that either lack of oxygen or the presence of nicotine around simple animals brings about structural changes resulting in death. A comparison, although a rough one, might

also be made between the fish eggs used by Loeb and the fat cells and œnocytes employed by the present writer. All these facts seem to indicate that either lack of oxygen or the presence of nicotine around the cells kills physically rather than chemically. The following paragraphs will considerably strengthen this statement.

It is well known that different fluids have different osmotic pressures, and this is also true for the blood of different animals. In order that tissues removed from various animals might be kept alive for some time, Lewis (15) has shown that they must be placed in fluids having different osmotic pressures. Endeavoring to make a fluid having an osmotic pressure equal to that of the blood in a grasshopper, Lewis used sea water, distilled water, grasshopper bouillon, sodium bicarbonate, and dextrose. The effect of osmotic pressure on cells is best illustrated by using red corpuscles. According to Cushny (6, p. 304–314), water passes into these cells readily and when placed into distilled water they swell up and burst, but when placed into an aqueous solution of sodium chlorid having an osmotic pressure greater than that of their contents, they shrink because the contained salts are unable to retain water against a higher concentration outside. A change brought about in the osmotic pressure of the blood might be a probable explanation of the death of the honeybees recently fed various salts by the present writer (18).

There are several theories regarding the manner in which drugs and powerful poisons affect the cells. Cushny has briefly summarized them about as follows: (1) Some drugs enter into definite chemical combinations with the constituent protoplasm; (2) some drugs act on the cells by changing the relation of the cell constituents in which they are dissolved; (3) some drugs alter the surface tension of the cells in relation to the surrounding fluids; (4) a few powerful drugs may act by altering the surfaces of the cells without penetrating into the interior; (5) many drugs may change the intracellular membranes; and (6) other drugs may reduce the permeability of the cellular membranes by altering their electric charges. Cushny says (6):

> From the present confusion the only legitimate conclusion seems to be that the activity of drugs depends on a large variety of factors and that pharmacological action can not be brought under any one law, either chemical or physical.

Shafer (21) has added another view which should be classified with the chemical ones, for it deals with the enzym-like cell constituents which accomplish oxidation. He thinks that contact insecticides, nicotine included, deleteriously affect the activities of the reductases, catalases, and oxidases in an unequal degree, thereby disturbing the natural or normal balance of the activities of these enzym-like factors.

SUMMARY

(1) Nicotine spray solutions do not pass into the tracheæ, nor do they penetrate the integuments of insects.

(2) The fumes from nicotine used as a fumigant, the vapors from nicotine spray solutions, and the odoriferous particles from evaporated nicotine spray solutions or from powdered tobacco pass into the tracheæ and are widely distributed to all the tissues.

(3) Regardless of how it is applied, whenever nicotine kills insects, as well as all other animals, it kills by paralysis, which in insects travels along the ventral nerve cord from the abdomen to the brain.

(4) The writer does not know just how nicotine paralyzes the nervous system, but he does know that it prevents the nerve cells from functioning, and that in regard to the simplest animals its presence around the cells causes the same structural changes resulting in death as observed when other animals of the same kind are deprived of oxygen. In such cases it seems to kill physically rather than chemically, but the evidence presented does not conclusively prove this view. In the higher animals it may kill by interfering with oxidation in the cells; whether this is accomplished physically or chemically the writer does not know, but concluding from the properties of nicotine he is inclined to attribute more to its physical effects than to its chemical effects.

LITERATURE CITED

(1) AUTENRIETH, Wilhelm.
1915. Laboratory Manual for the Detection of Poisons and Powerful Drugs. Ed. 4, translated by W. H. Warren. 320 p., 25 fig. Philadelphia.

(2) BAKER, A. C.
1915. The woolly apple aphis. U. S. Dept. Agr. Office Sec. Rpt. 101, 55 p., 3 fig., 15 pl. Literature referred to in the text, p. 53–55.

(3) BLYTH, A. W.
1895. Poisons: their Effects and Detection. Ed. 3, 724 p., illus. London.

(4) BRUNDAGE, A. H.
1910. A Manual of Toxicology ... Ed. 7, 428 p., illus., pl. New York, London.

(5) BUDGETT, S. P.
1898. On the similarity of structural changes produced by lack of oxygen and certain poisons. *In* Amer. Jour. Physiol., v. 1, no. 2, p. 210–214, 9 fig.

(6) CUSHNY, A. R.
1915. A Text-Book of Pharmacology and Therapeutics ... Ed. 6, 708 p., 70 fig. Philadelphia, New York.

(7) DEWITZ, J.
1912. The bearing of physiology on economic entomology. *In* Bul. Ent. Research, v. 3, pt. 4, p. 343–354. Bibliography, p. 352–354.

(8) FISCHER, Alfred.
1899. Fixirung, Färbung und Bau des Protoplasmas. 362 p., 21 fig., 1 col. pl. Jena. Literatur, p. 341–348.

(9) GREENWOOD, M.
1890. On the action of nicotin upon certain invertebrates. *In* Jour. Physiol., v. 11, suppl. no., p. 573–605.
(10) GUERCIO, Giacomo del.
1900. Sul potere mortifero dei liquidi alla nicotina e sull' uso di essi nella distruzione degli insetti. *In* Nuove Relaz. R. Staz. Ent. Agr. Firenze, s. 1, no. 3, p. 124–134.
(11) HOLLAND, J. W.
1905. A Text-Book of Medical Chemistry and Toxicology. 592 p., 108 fig., 8 pl. Philadelphia, London.
(12) KOBERT, Rudolf.
1906. Lehrbuch der Intoxikationen. Aufl. 2, Bd. 2. Stuttgart.
(13) KROCKER, Arthur.
1868. Ueber die Wirkung des Nikotins auf den thierischen Organismus. 39 p. Berlin.
(14) LANGLEY, J. N., and DICKINSON, W. L.
1890. Pituri and nicotin. *In* Jour. Physiol., v. 11, no. 4/5, p. 265–306. Papers quoted, p. 304–306.
(15) LEWIS, M. R.
1916. Sea water as a medium for tissue cultures. *In* Anat. Rec., v. 10, no. 4, p. 287–299, illus. Literature cited, p. 299.
(16) LOEB, Jacques.
1906. The Dynamics of Living Matter. 233 p., 64 fig. New York.
(17) MCINDOO, N. E.
1914. The olfactory sense of the honey bee. *In* Jour. Expt. Zool., v. 16, no. 3, p. 265–346, 24 fig. Literature cited, p. 345–346.
(18) ———
1916. The sense organs on the mouth-parts of the honey bee. Smithsn. Misc. Collect., v. 65, no. 14, 55 p., 10 fig. Literature cited, p. 52-54.
(19) PICTET, Amé, and ROTSCHY, A.
1904. Synthese des Nicotins. *In* Ber. Deut. Chem. Gesell., Jahrg. 37, Bd. 2, p. 1225–1235.
(20) SHAFER, G. D.
1911. How contact insecticides kill. I–II. Mich. Agr. Exp. Sta. Tech. Bul. 11, 65 p., 7 fig., 2 pl.
(21) ———
1915. How contact insecticides kill. III. Mich. Agr. Exp. Sta. Tech. Bul. 21, 67 p., 3 figs., 1 pl.
(22) SNODGRASS, R. E.
1910. The anatomy of the honey bee. U. S. Dept. Agr. Bur. Ent. Tech. Ser. 18, 162 p., 57 fig. Bibliography, p. 148–150.
(23) SOLLMANN, Torald.
1908. A Text-Book of Pharmacology ... Ed. 2, 1070 p., 127 fig. Philadelphia, London. Bibliographic register, p. 1029–1042.
(24) VAS, Friedrich.
1894. Zur Kenntniss der chronischen Nikotin- und Alkoholvergiftung. *In* Arch. Expt. Path. u. Pharmakol., Bd. 33, Heft 2/3, p. 141–154.
(25) WALLING, Eulalia V.
1906. The influences of gases and temperature on the cardiac and respiratory movements in the grasshopper. *In* Jour. Expt. Zool., v. 3, no. 4, p. 621–629.

PLATE 1

Fig. A.—Portion of the large longitudinal trachea of the house fly cut crosswise obliquely, showing the carmine acid "precipitate" *pr*. ×190. The fly had been submerged for one hour in a pure nicotine solution (1 : 500) colored with carmine acid.

Fig. B.—Combination drawing from two consecutive sections of a green peach aphis, showing the indigo-carmine "precipitate" *pr* in a trachea *tr*. ×500. The aphis had been submerged for 45 minutes in a pure nicotine solution (1 : 500) colored with indigo-carmine.

Fig. C.—Cross section of a large longitudinal trachea of larva of lesser wax moth, showing the indigo-carmine "precipitate" *pr* adhering to the tracheal wall *trw*. ×190. The larva had been submerged for 30 minutes in a pure nicotine solution (1:500) colored with indigo-carmine.

Fig. D.—Longitudinal section of one of the smallest tracheæ of the same larva as in figure C; same treatment and same enlargement.

Fig. E.—Longitudinal section of a large trachea and one of its branches of a coccid, showing the "precipitate" *pr* resulting from the union of pure nicotine and phosphomolybdic acid. ×500. The coccid had been submerged for 30 minutes in a pure nicotine solution (1 : 100).

Fig. F.—Portion of a cross section of an aphid (*Aphis rumicis*), showing the indigo-carmine "precipitate" *pr* in a spiracle *sp*. ×320. The aphid had been heavily sprayed with a pure nicotine solution (1 : 500) colored with indigo-carmine.

Fig. G.—Portion of a cross section of the same aphid as in figure F, showing no precipitate in the trachea *tr*, but much on the outside of the integument *int*. × 320.

Fig. H–O.—Longitudinal sections of spiracles *sp* with connecting tracheæ *tr*, showing how it is practically impossible for aqueous spray solutions to enter spiracles, owing to hairs *hr*, a closing plate *p*, and a peculiar arrangement of rims *r* at mouths of spiracles.

Fig. H.—Spiracle of a coccid (*Orthezia insignis*). × 500.

Fig. I.—Spiracle of a caterpillar of *Atteva aurea*. × 190.

Fig. J.—Spiracle of a larva of lesser wax moth (*Achroia grisella*). × 190.

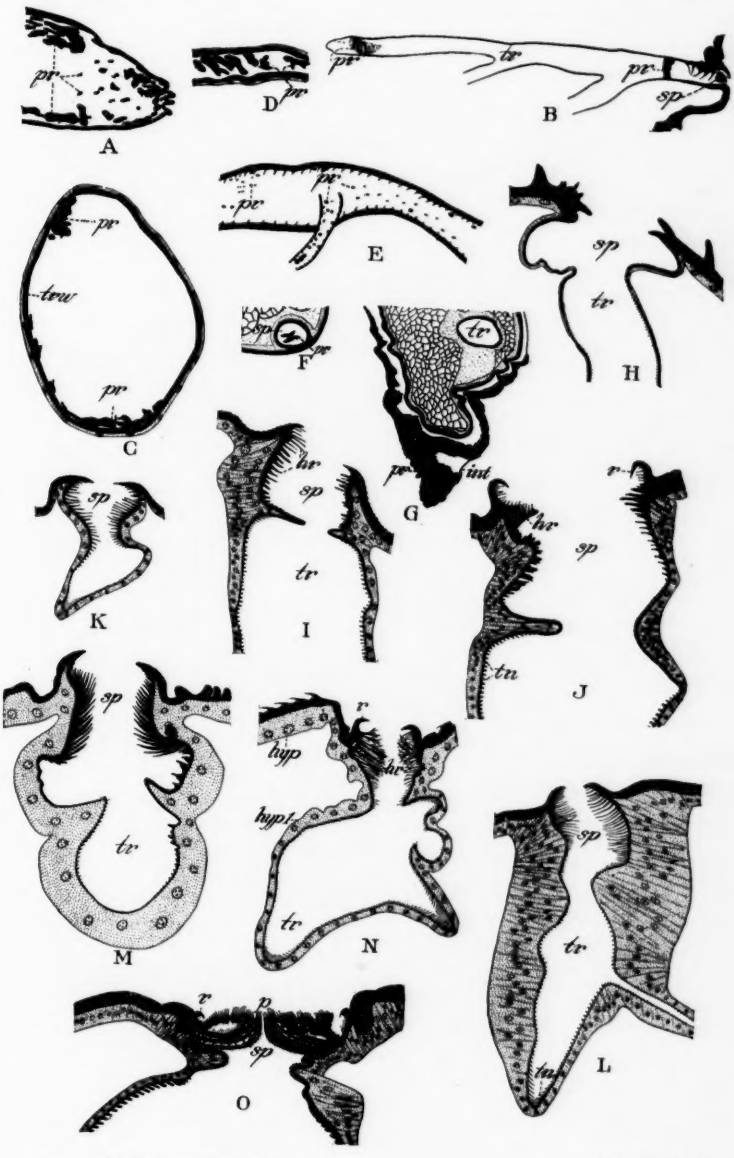
Fig. K.—Spiracle of a caterpillar of *Datana* sp. × 500.

Fig. L.—Spiracle of a caterpillar of a catalpa sphinx (*Ceratomia catalpae.*) × 500.

Fig. M.—Spiracle of a larva of a Colorado potato beetle (*Leptinotarsa decemlineata*). × 500.

Fig. N.—Spiracle of fall webworm (caterpillar of *Hyphantria cunea*). × 320.

Fig. O.—Spiracle of the tomato worm (larva of *Phlegethontius sexta*), showing the closing plate *p*. × 50.


A
D
B
C
E
F
H
G
K
I
J
M
N
L
O

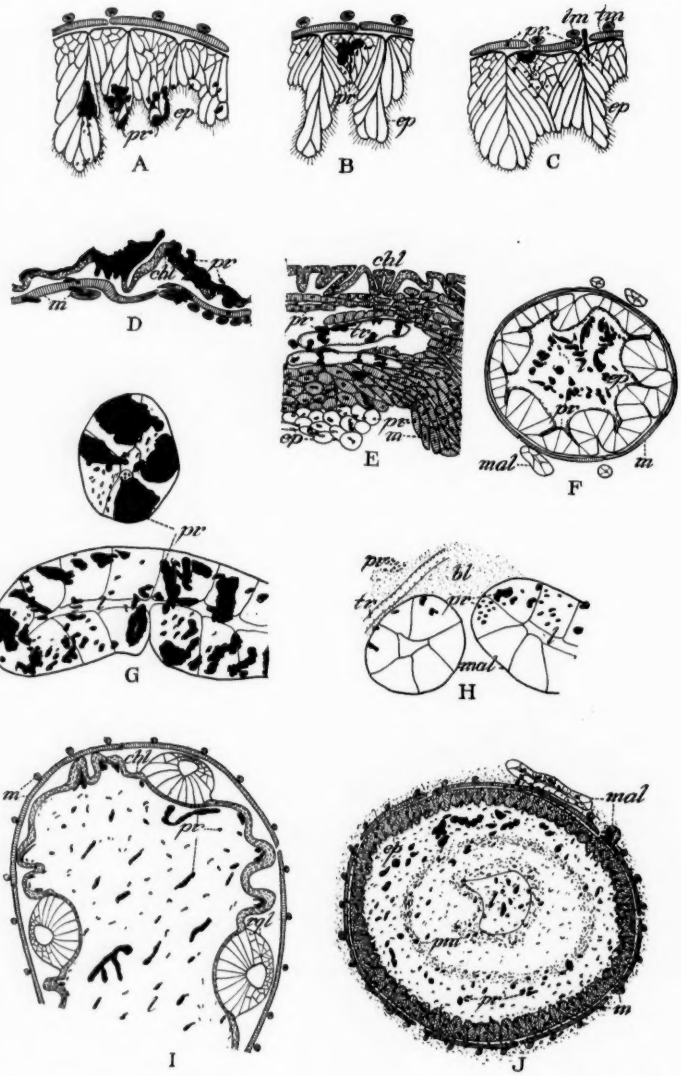

A
B
C
D
E
F
G
H
I
J
pr
ep
chl
m
tm
bl
mal
gl
pm

PLATE 2

Fig. A to J.—Cross sections of portions of the alimentary canals and Malpighian tubules of worker honeybees, showing "precipitated" indigo-carmine that had been fed with pure nicotine and honey to bees three days before they were fixed in absolute alcohol. Owing to poor fixation, most of cells are drawn diagrammatically.

Fig. A.—Portion of the wall of the ventriculus, showing the "precipitate" *pr* in inner ends of the epithelial cells *ep*. ×320.

Fig. B.—Portion of the wall of the ventriculus, showing the "precipitate" *pr* in the middle of the epithelial cells *ep*. ×320.

Fig. C.—Portion of the wall of the ventriculus, showing the "precipitate" in the outer ends of the epithelial cells *ep* and in the transverse muscle layer *tm*. ×320.

Fig. D.—Portion of the wall of the honey stomach joining the proventriculus, showing the "precipitate" *pr* in the chitinous *chl* and muscular *m* layers. ×320.

Fig. E.—Portion of the wall of the anterior part of the valve of the proventriculus, showing the "precipitate" *pr* in muscles *m*, tracheæ *tr*, and epithelial cells *ep*. ×320.

Fig. F.—Section through the small intestine, showing the "precipitate" *pr* in the center of the lumen *l* and lining epithelium *ep*, but none in the walls of this organ nor in the Malpighian tubules *mal* by it. ×50.

Fig. G.—Section through two Malpighian tubules *mal* against the ventriculus, showing the "precipitate" *pr* in their cells and lumens *l*. ×320.

Fig. H.—Section through two Malpighian tubules *mal* near the ventriculus, tracheal branch *tr* and blood *bl*, showing the "precipitate" *pr* in these tissues. ×320.

Fig. I.—Section of one-third of the rectum in a compressed condition, showing the "precipitate" *pr* in the lumen *l*, but none in the chitinous layer *chl*, nor in the rectal glands *rgl*, nor in the muscular *m* layer. ×50.

Fig. J.—Section through the middle of the ventriculus, showing the distribution of the "precipitate" *pr* in the lumen *l*, between the peritrophic membranes *pm*, in the epithelial *ep* and muscular *m* layers of the ventriculus and in the Malpighian tubules *mal*. ×50. The walls of the ventriculus were drawn diagrammatically.

PLATE 3

Fig. A to I, L, M.—Drawings and diagrams representing the distribution of precipitate resulting from the fumes of 40 per cent nicotine sulphate and phosphomolybdic acid. Figures A, B, F to I are from the same green peach aphis, and figures L and M are from the same house fly that had been fumigated. × 320. Figures D and E are diagrams, after Baker (2), representing the respiratory system of an aphid, *Eriosoma lanigerum*.

Fig. A.—Transverse-longitudinal section of a trachea of an aphid, showing the precipitate *pr* inside the trachea and in fat cells *fc* near by.

Fig. B.—Cross section of a portion of an aphid just molting, showing the precipitate *pr* on the outer surfaces of the old int_1 and the new integuments *int* and between them, but none in the fat cells *fc*.

Fig. C.—Combination drawing from six consecutive sections through thoracic ganglion of an aphid, showing the precipitate *pr* in three tracheal branches *tr* in the cortical layer *cl* and in the inner layer *il* of a ganglion.

Fig. D.—Diagram of the dorsal tracheal system of an aphid, showing the dorsal trunk *dt* and dorsal arch *da*.

Fig. E.—Diagram of the ventral tracheal system of an aphid, showing the anterior ventral arch *ava*, posterior ventral arch *pva*, and the ventral trunk *vt*.

Fig. F.—Combination drawing from five consecutive sections through the thorax of an aphid, showing the precipitate *pr* on the outer surface of the integument *int*, in the tracheæ *tr* and in the subesophageal ganglion *sg*.

Fig. G.—Portion of cross section of an optic lobe of an aphid, showing the precipitate *pr* inside and outside a tracheal branch *tr*.

Fig. H.—Cross section of the brain *br* and optic lobes *opl* of an aphid, showing the precipitate *pr* in the tracheal branch *tr* and in the cortical layer of the brain.

Fig. I.—Cross section of two ovaries *ov* of an aphid, showing the tracheal branch *tr* containing precipitate passing between them.

Fig. L.—Cross section of two tracheæ *tr* and a fat cell *fc* of a house fly, showing the precipitate *pr* in the tracheæ and in the fat cell outside its nucleus.

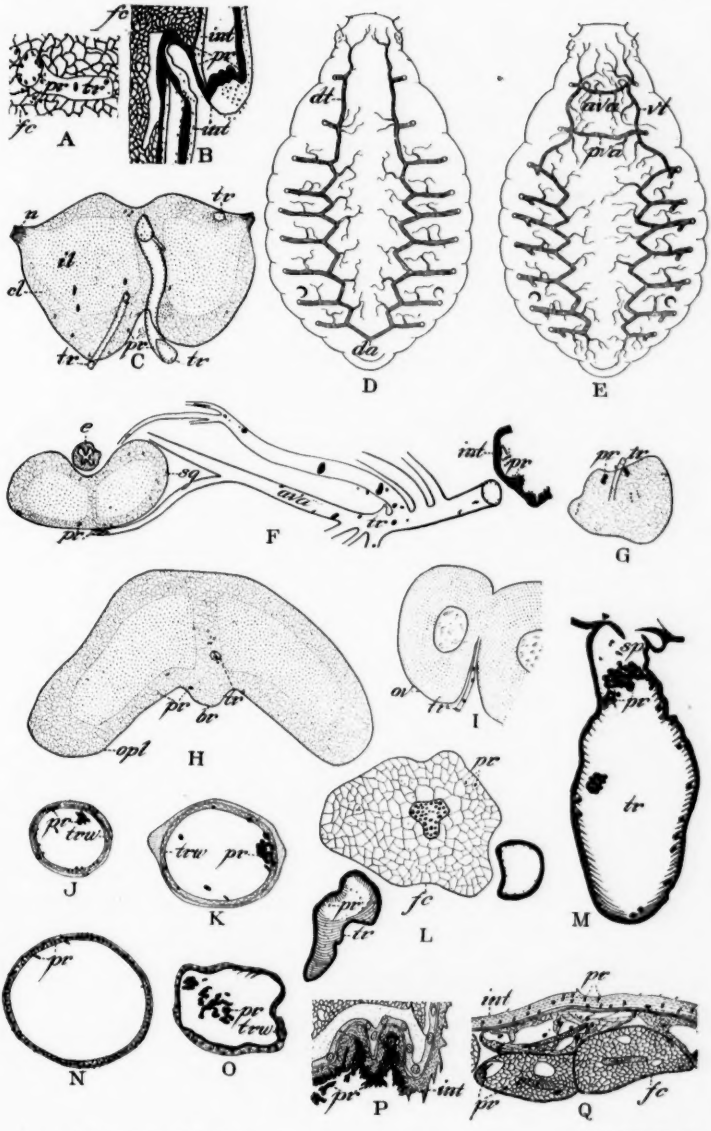
Fig. M.—Longitudinal section through a spiracle *sp* and its connecting trachea *tr* of a house fly, showing the precipitate *pr* in the neck of the spiracle and along the tracheal wall.

Fig. J and K.—Cross sections of the small tracheæ, showing the precipitate *pr* in newly formed tracheal walls *trw* resulting from the union of pure nicotine and phosphomolybdic acid. These and other insects had been submerged in pure nicotine for 35 minutes. × 190. Figure J is from a house-fly larva and figure K from a lesser wax-moth larva.

Fig. N and Q.—Cross sections, showing how well Carnoy's fluid passes through hard chitin, as indicated by remaining crystals *pr* of mercuric chlorid. Figure N is a trachea from a lesser wax-moth larva. × 190. Figure Q shows a portion of the integument *int* and the fat cells *fc* of an aphid, also showing the physical change in the fat cells caused by a fixative. × 500.

Fig. O.—Cross section of a medium-sized trachea of a lesser wax-moth larva, showing that pure nicotine did not pass into an older tracheal wall *trw* under the same conditions as stated for figure K. × 190.

Fig. P.—Cross section of portion of the integument *int* of an aphid (*Aphis rumicis*), showing that pure nicotine did not pass into chitin under same conditions as stated for figures J, K, and O. × 500.


fc
int
pr
tr
A
B
dt
da
D
avta
vt
pva
E
n
il
cl
C
e
sg
F
G
ov
I
H
opl
br
J
trw
K
L
M
sp
N
O
P
Q

ACIDITY AND ADSORPTION IN SOILS AS MEASURED BY THE HYDROGEN ELECTRODE

By L. T. SHARP and D. R. HOAGLAND,
Assistant Chemists, Agricultural Experiment Station of the University of California[1]

INTRODUCTION

The problem of soil reaction has come to occupy an increasingly important position in the realm of soil-fertility studies. This is evident from the numerous papers recently appearing upon the subject of soil acidity. The great diversity of opinion concerning the nature and methods of measuring soil acidity has served thus far only to confuse the matter. By adopting modern methods capable of measuring specifically the hydrogen ion,[2] data have been obtained by the writers which seem to offer a clearer conception of the question.

Before considering the question of soil acidity, it seems desirable to recall the fundamental significance of the terms "neutrality," "acidity," and "alkalinity." The dissociation of pure water produces H ions and OH ions in equal concentration, denoting neutrality. The product of the concentrations of these ions in any solution is a constant, approximately 1×10^{-14}. When the H ions are present in a concentration greater than the OH ions—that is, in a concentration greater than 1×10^{-7}, then the resulting solution is acid. Conversely, the presence of OH ions in greater concentration than 1×10^{-7} gives an alkaline solution. The term "acidity" is often construed to mean the total quantity of H ions which may be produced when the equilibrium is continually shifted by the introduction of OH-ions. This total acidity is referred to as potential acidity, while the H-ion concentration at any given moment determines the intensity of acidity, to use Gillespie's (13)[3] expression. In all probability excessive concentrations of H or OH ions in soil solutions exercise pronounced effects on plant growth and on the activities of soil bacteria.

Potential acidity or alkalinity may be due to undissolved substances or to soluble compounds only partly hydrolyzed or dissociated. An obvious illustration of this is afforded by the titration of a suspension of calcium carbonate in water. So long as solid calcium carbonate remains,

[1] From the Divisions of Soil Chemistry and Bacteriology and Agricultural Chemistry in equal cooperation. Acknowledgment is made to Mr. C. L. A. Schmidt for valuable suggestion during the progress of this work.

[2] To be more exact, according to recent physical-chemical views, it is the activity of the H ion, rather than the concentration, which is measured here. However, for the present purpose this distinction seems unimportant.

[3] Reference is made by number to "Literature cited," p. 143-145.

there will be a definite concentration of H and OH ions, while with the addition of acid the reaction of $H^+ + OH^- = H_2O$ occurs and equilibrium is restored by the solution of more calcium carbonate.

Soil acidity should not be set apart and considered as a phenomenon unrelated to the ordinary concepts of acidity. The use of such terms as "apparent acidity," "real acidity," "adsorption acidity" has led to a confusion of ideas, and, hence, should be discarded. With this in view, it seems desirable to emphasize the generally neglected point of view that the equilibria resulting in the production of H ions are in their essential nature the same for soil solutions as for other simpler chemical systems.

The methods now in vogue for estimating the lime requirement (soil acidity) have often proved to be unsatisfactory and in any case do not measure the intensity of acidity—that is, the H-ion concentration. In order to secure reliable data upon these points, the method of determining the H-ion concentration by means of a hydrogen electrode was used. Some preliminary experiments led to the adoption of a modification of Hildebrand's (17) apparatus, which will be described in detail later. Few similar measurements with soils have been previously recorded. The work of Saidel (29) was limited to a few alkaline soil extracts whose reaction had been changed by boiling. For reasons discussed later, data obtained on soil extracts are not convincing. Fischer (12) also made a few hydrogen-ion determinations with soil. In a more noteworthy investigation, Gillespie (13) has measured the H-ion concentration of 22 soils in water suspensions. The investigations reported in this paper were begun before the appearance of Gillespie's article. The present authors have extended the work into various other phases, such as titration with bases as a means of adjusting the soil reaction and of studying the general phenomena grouped under the term "adsorption." Moreover, various factors affecting the H-ion concentration of soil suspensions were also considered.

VARIOUS POINTS INVESTIGATED

In the course of these investigations several related questions were studied, in addition to the determinations of the H-ion concentration in various soil suspensions and in soil extracts. Experiments were undertaken in order to obtain further evidence regarding the influence of varying proportions of soil to water, grinding the soil, heating it at various temperatures, and of the addition of salts on the H-ion concentration. Consideration has also been given to the relation of HCO_3^-, CO_3^-, and CO_2 to soil reaction as measured by the electrometric method. Experimental data have likewise been secured with respect to the lime requirement of soils and the so-called "adsorption of bases." Finally, the apparatus and methods of procedure are described.

EXPERIMENTAL WORK

MATERIALS USED

Before taking up the detailed discussion of the data it appears desirable to describe briefly the materials used. Table I contains a list and description of all the soils referred to in this paper.

TABLE I.—*Description of soils used in experimentation*

Laboratory No.	Source of soil.	General type.	Remarks.
1	California	Silty clay loam	
2	do	do	
3	do	do	
4	do	do	
5	do	do	
6	do	do	
7	do	Fine sandy loam	
8	do	do	
9	do	do	
10	do	do	
11	do	do	Alkali soil.
12	do	Sandy loam	
13	do	Gravelly loam	
14	do	Clay adobe	
15	do	Fine sandy loam	Very infertile.
16	Wisconsin	Silty clay loam	
17	do	Sandy loam	
18	Pennsylvania	Silty loam	
19	California	Fine sandy loam	
20	Louisiana	Silty loam	
21	California	Peat	
22	Wisconsin	do	
23	California	Sandy loam	Alkali soil.
24	do	Fine sandy loam	Do.

HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS

Table II gives the amounts of soil and water used for making the suspensions. The soils were air-dried and passed through a 1-mm. sieve. The H-ion concentration is expressed in the customary units of gram molecules of H ion per liter.

In accordance with our preliminary statements, Table II gives evidence that soils may give rise to acid solutions—that is, solutions containing a preponderance of H ions over OH ions. This conception is in agreement with the conclusions which Truog (33) drew from his zinc-sulphid method. The work of Gillespie (13), paralleled by that presented in Table II, conclusively proves that there may be an excess of H ions in the solution bathing the soil particles.

Out of 22 soils examined by Gillespie (13), 17 were found to yield acid solutions. The writers experimented with 9 acid soils. The soils were of widely different types and origin. These facts do not support

the views of Cameron (5) concerning soil acidity, that it is generally due to the selective absorption of bases. They also are in opposition to the recent conclusions of Rice (26) and Harris (15), that water-soluble acids are not characteristic of acid soils.

TABLE II.—*Hydrogen-ion concentrations of suspensions of unground soil*

Soil No.	Quantity of soil.	Water.	Readings on voltmeter.	H-ion concentration (gram-molecules per liter).
	Gm.	*C. c.*		
1	2. 0	30	0. 763	0.4×10^{-7}
2	2. 0	30	. 753	$.6\times10^{-7}$
3	2. 0	30	. 759	$.5\times10^{-7}$
4	2. 0	30	. 763	$.4\times10^{-7}$
5	2. 0	30	. 759	$.5\times10^{-7}$
6	2. 0	30	. 753	$.6\times10^{-7}$
7	2. 0	30	. 761	$.5\times10^{-7}$
8	2. 0	30	. 752	$.6\times10^{-7}$
9	2. 0	30	. 752	$.6\times10^{-7}$
10	2. 0	30	. 742	$.9\times10^{-7}$
11	2. 0	30	. 760	$.5\times10^{-7}$
12	2. 0	30	. 770	$.3\times10^{-7}$
13	2. 0	30	. 550	$.2\times10^{-3}$
14	2. 0	30	. 753	$.6\times10^{-7}$
15	2. 0	50	*a*. 590	$.4\times10^{-4}$
15	15. 0	30	. 565	$.1\times10^{-3}$
15	. 1	50	. 627	$.9\times10^{-5}$
16	2. 0	50	*a*. 648	$.4\times10^{-5}$
16	5. 0	50	. 642	$.5\times10^{-5}$
16	10. 0	50	. 623	$.1\times10^{-4}$
16	15. 0	30	. 628	$.9\times10^{-5}$
16	. 1	50	. 619	$.1\times10^{-4}$
17	2. 0	50	*a*. 605	$.2\times10^{-4}$
17	5. 0	50	. 596	$.3\times10^{-4}$
17	10. 0	50	. 582	$.5\times10^{-4}$
17	25. 0	50	. 582	$.5\times10^{-4}$
17	2. 0	50	. 704	$.4\times10^{-6}$
18	15. 0	50	. 710	$.3\times10^{-6}$
18	2. 0	50	. 638	$.6\times10^{-5}$
19	. 1	50	. 638	$.6\times10^{-5}$
20	2. 0	50	*a*. 630	$.8\times10^{-5}$
20	5. 0	50	. 633	$.7\times10^{-5}$
20	10. 0	50	. 628	$.9\times10^{-5}$
20	15. 0	30	. 633	$.7\times10^{-5}$
20	5. 0	100	. 605	$.2\times10^{-4}$
21	2. 0	50	. 605	$.2\times10^{-4}$
22	5. 0	50	. 875	$.5\times10^{-9}$
23	5. 0	50	. 898	$.2\times10^{-9}$
24				

[a] Average of several determinations.

It is also evident from the data presented in Table II that there is a considerable range in the H-ion concentration of the soil suspensions. A number of soils known to be fertile show strikingly similar reactions, slightly alkaline, as indicated by an H-ion concentration of slightly less than 1×10^{-7}. Alkali soils, presumably containing sodium carbonate, show alkalinity corresponding to an H-ion concentration of 0.2×10^{-9}.

On the other hand, certain of the soils gave an H-ion concentration as high as 0.2×10^{-3}, which indicates considerable intensity of acidity.

Any ultimate conclusions regarding soil fertility must presuppose a knowledge of the composition of the soil solution. At present our methods do not enable us to study the soil solution itself, and consequently all deductions in regard to it must be purely inferential. In order to approximate the conditions existing in the soil solution, the ratio of water to soil in several cases was reduced as far as the method would permit. An inspection of the data indicates comparatively insignificant changes in the H-ion concentrations when widely varying proportions of water to soil are used. Most of these small fluctuations can be ascribed to the limitations of the apparatus. Hence, it is reasonable to assume that the H-ion concentrations of the soil suspensions approximate those of the soil solutions. This argument is theoretically sound, since in all the larger proportions of soil employed it is probable that the solution is saturated with respect to the acid-forming constituents.

RELATION OF HCO_3^-, CO_3^-, AND CO_2 TO SOIL REACTIONS

At present it has been found impracticable to simulate exactly the CO_2 equilibria existing under field conditions. In the case of several acid soils the partial saturation of the soil suspensions with CO_2 gas did not alter the reaction appreciably. Hence, it is quite possible that the CO_2 content of the soil solution may not materially modify the above conclusions regarding the magnitude of the H-ion concentration in acid soils. In other words, the soil acids, whether organic acids or acid silicates, are the chief factors determining the reaction.

On the contrary, the reaction of alkaline soils depends in large measure upon the equilibria between CO_2 gaseous, CO_2 dissolved, CO_3 ion, and HCO_3 ion.[1] From these considerations it might be predicted that the reaction of most alkaline soils is the resultant of the equilibria existing between HCO_3, CO_3, Ca ions and dissolved CO_2 in contact with the CO_2 of the soil atmosphere. From the work of Cameron and Bell (6) and Johnson (19) it seems proper to infer that HCO_3^- in this class of soils largely determines the reaction.

The application of the electrometric method to solutions or suspensions whose reaction depends upon the HCO_3 ion requires great precaution to prevent the decomposition of HCO_3^-, with a loss of CO_2. This reaction is slow, but there will be a gradual increase in the alkalinity of the solution as a result of the production of CO_3 ions. In the present work the shaking method by Gillespie (13) was employed and the decomposition of HCO_3^- largely avoided, although the results may tend to be slightly high.

[1] For a detailed discussion of this matter the reader is referred to Cameron and Bell (6) and to Johnson (19).

As a means of comparison the OH-ion concentration of solutions saturated with $Ca(HCO_3)_2$ and $CaCO_3$ were determined with an exactly similar technique. $Ca(HCO_3)_2$ gave a value for OH^- of 0.5×10^{-7} and for $CaCO_3$ 0.3×10^{-9}. The figure for $Ca(HCO_3)_2$ is almost identical with those obtained for the alkaline soils. This is in keeping with the theoretical considerations advanced above. T. Saidel (29) determined the OH-ion concentration of several soil extracts after prolonged boiling. His values approximate that given by $CaCO_3$, which is distinctly higher than the normal OH^- concentration for soils of this class.

At first attempts were made to estimate the H-ion concentration of filtered soil extracts. Serious difficulty was encountered in this procedure, in that the nitrates present were slowly reduced by the hydrogen gas in contact with platinum black. Obviously this reduction of nitrates would result in the production of NH_3 and a residue of fixed alkali, with a corresponding increase in the concentration of OH ions. While the reduction of nitrates does occur to some extent in soil suspensions, yet in this case the results are not appreciably changed. This is accounted for by the fact that the acid soils contain a large excess of potential acidity, while the absolute amounts of alkalinity produced are exceedingly minute. Therefore, equilibrium would be immediately restored without sensibly affecting the true H-ion concentration. As evidence thereof, the voltmeter readings for acid soils became constant within a few minutes and remained constant for an indefinite time.

In order to determine whether the reduction of nitrates gave rise to appreciable errors in the readings for alkaline soils, the OH^- concentration of a $Ca(HCO_3)_2$ solution was measured in the presence of large amounts of $NaNO_3$. No disturbance of the equilibria was noted under the conditions of the experiment. It has been suggested that the use of palladium or iridium, instead of platinum, for coating the electrodes would practically prevent the reduction of nitrates, but the authors have no experimental data upon this point.

While the reduction of nitrates prevents the use of the hydrogen electrode with extracts of acid soils, it is not believed that this factor would cause appreciable errors in the case of nutrient solutions containing nitrates. A nutrient solution would usually have a considerable potential acidity or alkalinity—for example, the unhydrolyzed or undissociated fraction of alkaline phosphates—so that constant results could be obtained, just as with soil suspensions. Moreover, by use of the shaking method described by Michaelis (21) the reduction of NO_3^- may be reduced to a minimum, but even this slight production of alkali causes serious error in soil extracts, where the total quantity of acid present is exceedingly small.

EFFECT OF GRINDING SOILS ON THE HYDROGEN-ION CONCENTRATION OF THEIR SUSPENSIONS.

The determination of the lime requirement of soils by the usual methods depends upon several factors. Among these factors the fineness of division has recently received some attention. Cook (9) found that in certain soils the lime requirement by the Veitch method increased with grinding, while Brown and Johnson (4) obtained opposite results working with another group of soils. Several of the soils already described were ground to pass a 200-mesh sieve. Table III shows the H-ion concentration of suspensions made from the ground soil.

TABLE III.—*Hydrogen-ion concentrations in suspensions of soil ground to pass through a 200-mesh sieve*

Soil No.	Quantity of soil.	Water.	Readings on voltmeter.	H-ion concentration (gram molecules per liter).
	Gm.	*C. c.*		
15	0.01	50	0.629	0.8×10^{-5}
15	.10	50	.617	$.1\times10^{-4}$
15	.50	50	.632	$.7\times10^{-5}$
15	1.0	50	[a].612	$.2\times10^{-4}$
15	2.0	50	[a].593	$.4\times10^{-4}$
15	5.0	50	[a].560	$.1\times10^{-3}$
15	10.0	50	.577	$.7\times10^{-4}$
16	1.0	50	[a].653	$.3\times10^{-5}$
16	5.0	50	.625	1.0×10^{-5}
17	.01	50	[a].615	$.1\times10^{-4}$
17	1.0	50	.624	$.1\times10^{-4}$
17	5.0	50	[a].597	$.3\times10^{-4}$
20	2.0	50	[a].623	$.1\times10^{-4}$
19	1.0	30	.646	$.4\times10^{-5}$
19	2.0	50	[a].634	$.7\times10^{-5}$
18	.5	50	.750	$.7\times10^{-7}$
18	2.0	50	.748	$.7\times10^{-7}$
18	5.0	50	.762	$.4\times10^{-7}$

[a] Average of several determinations.

By comparing Table III with Table II it will be seen that with the exception of soil 18 grinding did not materially alter the H-ion concentration of the soil suspensions. These remarks bear no reference to the lime requirement, which will be discussed later, but apply only to intensity of acidity. Apparently the anomalous behavior of soil 18 may be explained on the supposition that the interior cores of the soil particles are of a different composition from the exterior, partially weathered layers. This hypothesis may also account for the findings of Brown and Johnson (4).

The data of Table III also corroborate in the main those presented in Table II in reference to the slight fluctuations in H-ion concentration due to varying the proportion of soil to water.

EFFECT OF HEATING ON THE HYDROGEN-ION CONCENTRATION

In order to ascertain the effect of heating upon the H-ion concentration, several soils were subjected to the heating treatments indicated in Table IV.

TABLE IV.—*Effect of heating on H-ion concentration of soil suspensions*

Soil No.	Quantity of soil.	Water.	Method of heating.	Time of heating.	Voltmeter reading.	H-ion concentration (gram molecules per liter).
	Gm.	*C. c.*		*Hours.*		
15	2	50	Oven 140° C.	3	0.578	0.6×10^{-4}
16	2	50	do.	3	.621	$.1\times10^{-4}$
17	2	50	do.	3	.583	$.5\times10^{-4}$
17	5	50	Muffle below red heat.	1	.652	$.3\times10^{-5}$
17	2	50	do.	1	.730	$.1\times10^{-6}$
17	2	50	Blasted	1	.812	$.6\times10^{-8}$
19	2	50	Oven 140° C.	3	.603	$.2\times10^{-4}$
19	2	50	do.	3	.598	$.3\times10^{-4}$
20	2	50	do.	3	.672	$.2\times10^{-5}$
20	2	50	Muffle below red heat.	3	.678	$.1\times10^{-5}$
20	2	50	Blasted	3	.803	$.8\times10^{-8}$

The data of Table IV confirm the views of Connor (8), in that the intensity of acidity decreases when the soils are heated at high temperatures. The insufficiency of the data concerning heating at 140° C. does not admit of positive conclusions, though there is indication that the H-ion concentration may be slightly increased by this treatment.

ESTIMATION OF THE LIME REQUIREMENT BY THE ELECTROMETRIC METHOD

The rational treatment of acid soils requires that sufficient lime be added to bring the soil to a neutral or slightly alkaline reaction. The attainment of this point may be definitely determined by the method described in this paper. Many empirical methods have been suggested for the determination of the lime requirement, but the inaccuracy of these methods is indicated by the enormous variations in results, as shown in the comparative tests reported by Ames and Schollenberger (1) and others. These findings are also confirmed by data obtained in this laboratory.

An attempt was made to determine more precisely the lime requirement of soils by a method of electrometric titration with calcium hydroxid, $Ca(OH)_2$, in which a standard calcium-hydroxid solution was added to the soil suspensions until a definite alkaline reaction was obtained. The data were supplemented by pot and beaker studies. Tables V and VI present the results of these experiments.

TABLE V.—*Titration of soil suspensions with calcium hydroxid for lime requirement*

Soil No.	Quantity of soil.	Water.	Quantity of calcium hydroxid added (calculated to calcium carbonate).		H-ion concentration.				Time of titration.
					Original.		After titration.		
			Entire quantity.	Per gram of soil.	Voltmeter reading.	Gram molecules per liter.	Voltmeter reading.	Gram molecules per liter.	
	Gm.	*C. c.*	*Gm.*	*Gm.*					*Hours.*
15.....	5	50	0.0085	0.0017	0.590	0.4×10^{-4}	0.775	0.2×10^{-7}	3
15.....	5	50	.0128	.0026	.590	$.4\times10^{-4}$	.796	$.1\times10^{-7}$	44
16.....	2	50	.0054	.0027	.654	$.3\times10^{-5}$	.761	$.4\times10^{-7}$	26
17.....	2	50	.0061	.0030	.611	$.2\times10^{-4}$	.759	$.5\times10^{-7}$	26
17.....	2	50	.0070	.0035	.599	$.3\times10^{-4}$	.757	$.5\times10^{-7}$	5
19.....	2	50	.0081	.0040	.638	$.6\times10^{-5}$	.770	$.3\times10^{-7}$	25
20.....	2	50	.0053	.0026	.618	$.1\times10^{-4}$	.754	$.6\times10^{-7}$	3
20.....	2	50	.0047	.0023	.623	$.1\times10^{-4}$	.757	$.5\times10^{-7}$	5
18.....	2	50	.00068	.00034	.707	$.4\times10^{-6}$	.758	$.5\times10^{-7}$	20

TABLE VI.—*Results of beaker and pot studies*

Soil No.	Soil in beaker or pot.	Total quantity of calcium carbonate added.	Calcium carbonate per gram of soil.	Treated soil tested.	Water.	Reaction of treated soil.		Remarks.
						Readings on voltmeter.	H ion (gram molecules per liter).	
	Gm.	*Gm.*	*Gm.*	*Gm.*	*C. c.*			
15..	200	0.200	0.001	5	50	0.664	0.2×10^{-5}	Incubated 7 days at 30° C.
15..	200	.400	.002	5	50	.799	$.9\times10^{-8}$	Do.
15..	11,200	10.0	.0009	5	50	.630	$.8\times10^{-5}$	In jar 2 months.
15..	11,200	15.0	.0013	5	50	.733	$.1\times10^{-6}$	Do.
16..	50	.135	.0027	5	50	.752	$.6\times10^{-7}$	Incubated 7 days at 30° C.
17..	25	.090	.0036	5	50	.728	$.2\times10^{-6}$	Do.
17..	50	.250	.0050	5	50	.777	$.2\times10^{-7}$	Do.
18..	50	.017	.00034	5	50	.740	1.0×10^{-7}	Do.
19..	25	.100	.0040	5	50	.755	$.5\times10^{-7}$	Do.
20..	25	.065	.0026	5	50	.780	$.2\times10^{-7}$	Do.

Such a method is logically adapted to obtain the information necessary for the proper adjustment of the soil reaction by the addition of lime. There are, however, certain difficulties met with in its application to soils. One of the chief obstacles is due to the relative insolubility of the acid-forming constituents of soils, which prevents a rapid attainment of equilibrium. This drawback can probably be overcome by the use of a shaking machine. Another but presumably minor source of error lies in the loss of CO_2 from the soil suspension, as previously mentioned.

In order to determine whether the titrations with calcium hydroxid might serve as a guide for the application of lime necessary to produce a neutral or slightly alkaline reaction several beaker and pot studies were undertaken. A reference to Tables V and VI shows that in these soils approximate neutrality resulted from the admixture of calcium carbon-

ate with the soil in amounts indicated by the titrations. While these data are not extensive, a valuable correlation is suggested.

So far as the writers are aware, this is the first time that this electrometric titration has been applied to soil studies. It is believed that with further work a valuable means may be developed for the more exact determination and adjustment of soil reaction. Hence, this method may be extremely useful in the accurate control of soil reaction in many field and pot experiments. The somewhat complicated nature of the apparatus and the time involved would doubtless militate against its general adoption for routine analyses.

EFFECT OF THE ADDITION OF NEUTRAL SALTS ON THE HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS

The effect on the H-ion concentration of soil suspensions produced by the addition of neutral salts is a matter of considerable theoretical interest and of practical importance. Such data may be of significance in their relation to the application of soluble fertilizing salts and to the various lime-requirement methods dependent upon treatments with solutions of potassium nitrate. The desirability of similar measurements as correlated with the effects of soluble salts on the physical condition of soils has already been suggested by one of the authors (31). The bearing of the results upon adsorption phenomena will be discussed in a later section of this paper.

Table VII records the changes in H-ion concentration of various soil suspensions when treated with different neutral salts:

TABLE VII.—*Effects of neutral salts on H-ion concentration of soil suspensions*

Soil No.	Quantity of soil.	Water.	Salt added.	Quantity of salt.	Original soil.		Treated soil.	
					Voltmeter reading.	H-ion (gram molecules per liter).	Voltmeter reading.	H-ion (gram molecules per liter).
	Gms.	*C. c.*		*Gm.*				
16..	2	30	Potassium chlorid.	1	0.628	0.9×10^{-5}	0.582	0.5×10^{-4}
16..	2	30	Sodium chlorid...	1	.628	$.9\times10^{-5}$	.572	$.8\times10^{-4}$
16..	2	30	Barium chlorid...	1	.628	$.9\times10^{-5}$	.555	$.2\times10^{-3}$
20..	2	30	Potassium chlorid.	1	.639	$.5\times10^{-5}$	.575	$.7\times10^{-4}$
20..	2	30	Sodium chlorid...	1	.639	$.5\times10^{-5}$	.568	1.0×10^{-4}
20..	2	30	Barium chlorid...	1	.639	$.5\times10^{-5}$	.564	$.1\times10^{-3}$
15..	2	30	Potassium chlorid.	1	.598	$.3\times10^{-4}$	.548	$.2\times10^{-3}$
15..	2	50	Sodium chlorid...	0.5	.598	$.3\times10^{-4}$	.551	$.2\times10^{-3}$
15..	2	30	Barium chlorid...	1	.598	$.3\times10^{-4}$	.535	$.3\times10^{-3}$
18..	2	30	Potassium chlorid.	1	.690	$.7\times10^{-6}$	.614	$.2\times10^{-4}$
18..	2	30	Sodium chlorid...	1	.690	$.7\times10^{-6}$	.615	$.2\times10^{-4}$
18..	2	30	Barium chlorid...	1	.690	$.7\times10^{-6}$	.590	$.4\times10^{-4}$
14..	2	30	Potassium chlorid.	1	.753	$.6\times10^{-7}$	.662	$.2\times10^{-5}$
14..	2	30	Sodium chlorid...	1	.753	$.6\times10^{-7}$	.672	$.2\times10^{-5}$
14..	2	30	Barium chlorid...	1	.753	$.6\times10^{-7}$	.654	$.3\times10^{-5}$
1...	2	50	Potassium chlorid.	0.5	.763	$.4\times10^{-7}$	.763	$.4\times10^{-7}$

A study of the foregoing data makes it evident that in all cases there has been a distinct increase in the H-ion concentration, when either potassium chlorid, sodium chlorid, or barium chlorid was added to the suspension in the quantities indicated. The increase does not vary greatly for the three salts used, but on the whole the barium chlorid has a somewhat greater effect. In soil 14 we have an interesting case in which a change of reaction from alkaline to acid has taken place, as a result of the addition of neutral salts of sodium, potassium, or barium. It is obvious that such a soil would probably be adjudged acid by the potassium-nitrate method, although its normal reaction is, in fact, slightly alkaline. In a more normal type of soil (No. 1), however, no change in the reaction is found. The use of calcium chlorid in the above experiment was found to be impracticable on account of the difficulty in obtaining a perfectly neutral salt.

ADSORPTION OF OH IONS BY SOILS IN SUSPENSIONS OF VARIOUS BASES

So far the H-ion concentration of soil suspensions, the factors affecting it, and the possible use of an electrometric titration method for determining the lime requirement have been the chief topics considered. We shall now consider another phase of the general problem, involving the question of the adsorption of OH ions by the soil. The hydrogen electrode has proved useful for this purpose.

Changes of the OH-ion concentration of soil suspensions were measured when varying quantities of different hydrates were added. In addition the removal of Ca from a solution of hydrate in contact with two of the soils studied was noted. The data obtained from these experiments are incorporated in Tables VIII and IX.

To suspensions of soils that pass through a 200-mesh sieve the hydrates were added, a small portion at a time, until the neutral point was just passed; then further additions of hydrate were made until an arbitrarily selected OH-ion concentration was maintained over a considerable period of time, as noted in Table VIII. After each addition of the hydrate the soil suspension was given a prolonged shaking. The bases added have been calculated for convenience of comparison to the equivalent OH expressed in grams. The above data enable us to estimate the approximate quantity of OH ions removed from the solution by the soil, and in two cases where calcium hydroxid was added the removal of Ca has also been determined by the usual analytical method.

TABLE VIII.—*Results of titrations of ground (200-mesh sieve) soil with various bases*

Soil No.	Quantity of soil.	Water.	Base.	Weight of OH.	OH per gram of soil.	Time of titration.	Original OH-ion concentration (gram molecules OH per liter).	OH-ion concentration at approximate neutrality (gram molecules OH per liter).	Base added beyond neutral point (weight of OH).	OH per gram soil.	Time of titration.	Final OH-ion concentration (gram molecules OH per liter).
	Gm.	*C.c.*		*Gm.*	*Gm.*	*Hrs.*			*Gm.*	*Gm.*	*Hr.*	
15..	0.5	50	Calcium hydroxid.	0.00038	0.00076	1.5	0.9×10^{-9}	0.8×10^{-7}	0.00006	0.00012	3	1.9×10^{-7}
15..	10.0	50	.....do.....	.00798	.00079	2.0	$.9\times10^{-10}$	2.0×10^{-7}	.00088	.00009	96	2.9×10^{-7}
15..	1.0	50	.....do.....	.....	.....	.....	3.9×10^{-10}	.....	[a].00211	[a].00211	26	$.9\times10^{-4}$
15..	1.0	50	Sodium hydroxid.	.....	.....	.....	3.6×10^{-10}	.....	[a].00207	[a].00207	26	2.2×10^{-4}
15..	2.0	50	Calcium hydroxid.	.00163	.00081	.....	2.1×10^{-10}	4.3×10^{-8}	.00014	.00007	3	2.7×10^{-7}
15..	2.0	50	Sodium hydroxid.	.00131	.00065	24.0	1.5×10^{-10}	$.7\times10^{-7}$	.00026	.00013	27	2.4×10^{-7}
15..	5.0	50	Calcium hydroxid.	.00429	.00085	6.0	$.7\times10^{-10}$	$.9\times10^{-7}$	.00029	.00006	50	1.1×10^{-7}
15..	5.0	50	Sodium hydroxid.	.00350	.00070	3.0	4.2×10^{-11}	1.1×10^{-7}	.00052	.00010	27	1.2×10^{-7}
16..	5.0	50	.....do.....	.00560	.00112	20.0	$.6\times10^{-9}$	$.6\times10^{-7}$	.00105	.00021	42	1.7×10^{-7}
16..	1.0	50	Calcium hydroxid.	.00103	.00103	24.0	2.0×10^{-9}	$.6\times10^{-7}$	.00340	.00340	48	4.2×10^{-5}
16..	1.0	50	Barium hydroxid.	.00099	.00099	0.5	1.0×10^{-9}	1.9×10^{-7}	.00280	.00280	30	3.8×10^{-5}
16..	1.0	50	Sodium hydroxid.	.00105	.00105	3.0	2.0×10^{-9}	1.5×10^{-7}	.00263	.00263	30	4.2×10^{-5}
16..	1.0	50	Potassium hydroxid.	.....	.....	.....	1.9×10^{-9}	.....	[a].00324	[a].00324	24	5.7×10^{-5}
17..	1.0	50	Calcium hydroxid.	.00088	.00088	2.5	5.8×10^{-10}	1.9×10^{-7}	.00474	.00474	30	$.7\times10^{-4}$
17..	1.0	50	Barium hydroxid.	.00066	.00066	1.5	6.3×10^{-10}	1.7×10^{-7}	.00406	.00406	30	$.9\times10^{-4}$
17..	5.0	50	Calcium hydroxid.	.00650	.00130	1.0	2.0×10^{-10}	$.8\times10^{-7}$	.00800	.00160	22	1.3×10^{-5}
17..	5.0	50	Sodium hydroxid.	.00438	.00087	4.0	2.2×10^{-10}	$.8\times10^{-7}$	.00526	.00105	110	1.7×10^{-5}
19..	2.0	50	Calcium hydroxid.	.00306	.00153	7.0	$.9\times10^{-9}$	$.9\times10^{-7}$	.01010	.00505	30	4.2×10^{-5}
19..	2.0	50	Barium hydroxid.	.00330	.00165	21.0	$.9\times10^{-9}$	$.8\times10^{-7}$	.00858	.00429	30	3.5×10^{-5}
19..	2.0	50	Sodium hydroxid.	.00207	.00103	7.0	$.7\times10^{-9}$	1.0×10^{-7}	.00350	.00175	30	4.1×10^{-5}
19..	2.0	50	Potassium hydroxid.	.00198	.00099	24.0	1.3×10^{-9}	$.7\times10^{-7}$	.00524	.00262	30	3.8×10^{-5}
20..	2.0	50	Calcium hydroxid.	.00118	.00059	4.0	5.8×10^{-10}	5.9×10^{-8}	.00446	.00223	28	4.8×10^{-5}
20..	2.0	50	Sodium hydroxid.	.00091	.00045	3.5	6.3×10^{-10}	$.7\times10^{-7}$	.00249	.00125	28	3.1×10^{-5}

[a] Includes base added to neutralize acid.

TABLE IX.—*Removal of calcium from solution of calcium hydroxid by soils*

Experiment No.	Soil No.	Weight of soil.	Water.	Calcium added.	Calcium recovered in solution.	Calcium removed from solution.	
		Gm.	*C.c.*	*Gm.*	*Gm.*	*Gm.*	*P. ct.*
1	17	20	50	0	0.0003	.....	.....
2	17	20	50	.0055	.0007	0.0048	87
3	17	20	50	.0137	.0023	.0114	83
4	17	20	50	.0192	.0053	.0139	73
5	17	20	50	.0220	.0067	.0153	70
6	17	20	50	.0247	.0089	.0158	64
7	20	20	50	.....	.0003	.....	.....
8	20	20	50	.0022	.0003	.0019	86
9	20	20	50	.0038	.0003	.0035	92
10	20	20	50	.0055	.0005	.0050	91
11	20	20	50	.0066	.0005	.0061	92
12	20	20	50	.0082	.0009	.0073	89

When 0.1 mgm. of OH as calcium hydroxid is added to 50 c. c. of distilled water, there is produced an OH-ion concentration of 3.8×10^{-5} gram molecules per liter. To reach this same concentration of OH ions in the presence of the soil requires the addition of a much greater quantity of hydrate, whether of sodium, calcium, barium, or potassium. The difference between the quantity of hydrate necessary to add to distilled water to obtain the concentration fixed upon and that required when the soil is present gives an index of the amount of hydroxyl ions removed from the ionic equilibrium. In the case of acid soils it is probable that until the neutral point is reached the removal of OH ions can be accounted for by the reaction between the added OH ions and H ions derived from the soil acids. Beyond the neutral point, however, another type of reaction must necessarily be involved. For example, soil 16 required the addition of 1.0 mgm. of OH as calcium hydroxid per gram of soil to give the suspension an OH-ion concentration of 0.6×10^{-7}, while the second point, representing a concentration of 4.2×10^{-5} gram molecules of OH ions per liter, required the further addition of 3.4 mgm. of OH per gram of soil. By subtracting the quantity of OH required to reach the same point when added to distilled water, it is evident that 3.3 mgm. of OH have been removed by some mechanism not associated with the neutralization of acid. The nature of the latter type of reaction has been the subject of much conjecture; but before entering upon a detailed discussion of this matter it is desirable to point out certain other relations which may be derived from a further study of the data presented above. These are concerned with the comparison of the amounts of OH ions removed from solutions of the various bases, when added in combination with different positive ions. In general, it may be said that to bring about this higher OH-ion concentration in the soil suspension requires a larger equivalent of calcium hydroxid and barium hydroxid than of sodium hydroxid or potassium hydroxid. As a striking instance of this, soil 19 may be cited. In order to assure a reasonable degree of validity for these comparisons, they have been made, in accordance with Hanley's (14) suggestions, under conditions such that the suspended soil particles were in equilibrium with solutions of the same concentration. It is difficult to draw definite conclusions in regard to the relative quantities of bases required to produce neutrality, but it may be said that the equivalent quantities are of approximately the same magnitude. Exact equivalents would hardly be expected on account of various side reactions involving the interchange of bases.

The interesting observation recorded in Table VII that barium chlorid when added to soils brings about a slightly greater acidity than when sodium or potassium chlorid is added may possibly be correlated with the fact that a greater quantity of OH is removed by the soil when added as barium hydroxid than when added as sodium or potassium hydrate. This agrees with certain contentions of Parker (24).

Many investigators consider soil acidity as being primarily related to adsorptive phenomena. Cameron (5) has explained the reddening of blue litmus paper by many soils as due to selective absorption of the base from the litmus base and does not correlate the reddening of the litmus paper with the presence of soluble acid except in very rare cases. Harris (15) also ascribes soil acidity to the selective adsorption of bases by the soil. Bogue (3) and others have expressed a similar opinion concerning soil acidity. The phenomena of adsorption, as is well known, has also been invoked to explain the fixation of various ions by the soil.

Another view of soil acidity attributes it to an exchange of bases in which a weak base, as aluminium, has been replaced by a strong base such as potassium. The resulting hydrolysis of the aluminium salts produces an acid reaction in the solution. This view has been advanced most recently by Rice (26), Conner (8), Loew (20), Daikuhara (10), and Veitch (35). This is a plausible explanation for the acidity of soils which have been treated with salts, although it does not indicate the reaction of the soil previous to the salt treatments. The results already presented in regard to the addition of salts show that in the case of acid soils this treatment markedly increased the H-ion concentration in every case. One slightly alkaline soil was also found to give an acid reaction after the salt treatments. These data are in direct accord with the accumulated evidence concerning the acidity developed when a strong base reacts with the soil constituents replacing a weak base. In the light of these observations it is quite unnecessary to have recourse to adsorption theories to account for the acidity of many salt-treated soils, even though the soils originally might have had an alkaline reaction. This is illustrated in the case of soil 14, already cited. Obviously, the principal reactions involved in producing the acidity in such cases are of a chemical rather than of a physical nature. The importance of such an exchange of bases is emphasized by the work of Sullivan (32), Rice (26), and Conner (8).

Truog (33), Hanley (14), Gillespie (13), and Loew (20) have recognized that the acidity of a soil is due to the presence of soluble acids. The experimental evidence reported in this paper has brought the writers to a similar conclusion, in which case it is not necessary to associate soil acidity with physical adsorption.

A great many obscure phenomena in the fields of biochemistry and soil chemistry, as well as many others, have been classified under the indefinite terms "absorption" and "adsorption." The indiscriminate use of these terms has not served to clarify the problem involved. Thus, the fixation of plant foods by soils has been explained by some investigators entirely on the basis of physical adsorption. Frequently, the importance of the chemical exchange of bases has been disregarded. Moreover, the possibility of addition compounds as suggested by Sullivan (32) has not received sufficient consideration. The results given in Table VII show

that the OH ions of a solution of calcium hydroxid have been removed in large measure from the solution by the soil, while those in Table VIII demonstrate a simultaneous loss of calcium from the solution. Evidently both the Ca and OH ions have been removed to a notable extent from the solution by the soil. Two explanations of these phenomena suggest themselves. One explanation would assume a condensation of the calcium hydroxid as a whole on the surfaces of the soil particles. The other, in accordance with the ideas of Sullivan (32), Van Bemmelen (34), and Lemberg (32, p. 20–23), considers that the calcium hydroxid has been chemically united with some of the soil constituents, forming direct addition compounds. In view of the evidence embodied in the literature, the second theory seems the more tenable. In many ways the chemical explanation appears to be more logical in accounting for the fixation of bases by soils.

Moreover, the theories which regard adsorption in soils as a chemical phenomenon have received an element of support in the proposed chemical structure of the silicates. Clarke and Steiger (7) have shown that the composition of the silicates is such that it frequently admits of an exchange of bases. This has also been recognized by soil chemists. Likewise, from the structural formulæ for silicates proposed by the above investigators, it is evident that acid salts of the various silicic acids might contain replaceable hydrogen. Loew (20) attributes the acidity of certain Porto Rican soils to the presence of acid silicates. As previously mentioned, Sullivan (32) has also pointed out that a basic hydrate may form an addition compound with silicates.

The theory of selective adsorption of a single ion which has been advanced by various investigators is in its final analysis not entirely comprehensible. One objection to this theory lies in the disregard of the ionic equilibrium. For example, it is frequently assumed that from a solution of a neutral salt one ion may be withdrawn by a colloid independently of its equivalent, oppositely charged ion. Thus if K^+ be selectively removed from a solution of potassium chlorid (KCl), then the above assumption may be diagramatically represented as follows:

$$\begin{array}{l} (\text{Colloid}-\text{K})+\overset{-}{\text{Cl}}+\text{H}_2\text{O} \\ \qquad\qquad\qquad\quad \uparrow\downarrow \\ \qquad\qquad\qquad\quad \overset{+}{\text{H}}+\overset{-}{\text{OH}} \end{array}$$

Obviously, such a system is electrically unbalanced. In order to meet this difficulty, the electrical double-layer theory of Helmholtz has been proposed. A modification of this theory has been especially urged by Billitzer (2). In its simplest form the electrical double-layer theory assumes that one ion may become more closely associated with a colloidal particle than the oppositely charged ion. The force which binds the ion to the colloid is not well understood, but there is some justification for believing that the most closely associated ion imparts its

charge to the colloidal particle. The charge thus produced on the surface of the particle is balanced by oppositely charged ions in the immediate sphere of attraction. In this manner the conditions of electrical equilibrium are accounted for.

Parker (24) has avoided some of the difficulties involved in explaining the electrical equilibria on the assumption that one ion alone is removed by a colloid. He claims that potassium chlorid in aqueous solution is hydrolyzed, yielding hydrochloric acid and potassium hydroxid. The latter is then considered to be withdrawn from the solution as a whole. Obviously, this does not explain the mechanism by which the potassium hydroxid is combined with the colloid, although it does take into account the electrical equilibria and the presence of acid in the solution. It does not, however, exclude the possibility of a direct chemical addition product of potassium hydroxid with some of the soil constituents.

Either of the last two theories may account for the presence of acid in the filtrate or supernatant liquid derived from a colloid in contact with a salt solution. Whichever explanation may be preferable, for practical purposes the final result is the same—that is, the colloid has retained equivalent quantities of negative and positive ions, and chemically equivalent quantities remain in solution.

Since these theories of adsorption necessitate the removal of both positive and negative ions, the usefulness of the idea of selective adsorption as applied to many types of soil reactions may be questioned, especially in view of the possibility that chemical reactions, at least to a considerable extent, may account for the observed phenomena.

This statement is not to be construed as denying the probability of a partial condensation of a chemical compound on solid surfaces. A clear exposition of this type of phenomena is given by Patten (25). The magnitude of such condensations is exceedingly variable, for it depends upon the physical conditions of the particular system, and, hence, it is difficult to estimate its significance in any specific instance. Indeed, Robertson (27) is inclined to believe that surface condensation accounts for only a small portion of the total amount of substance combined with the adsorbing body. In his opinion most reactions designated by the terms "absorption" and "adsorption" obey the usual laws formulated for chemical reactions. The influence of surface in accelerating and possibly changing the nature of these reactions is not denied. This solution of the problem, as stated by Robertson, is not admitted by certain other investigators, notably Wolfgang Ostwald (23). Apparently, no final decision on this matter has been reached by physical chemists.

It has been the aim of the present paper to set forth certain results and methods of investigation which may serve to throw additional light on some of the unsolved problems of soil fertility. Especially has the method of attack made possible an investigation of the important ques-

tions of soil acidity and adsorption on a new basis. The evidence has been confirmatory of the view that soil acidity is fundamentally dependent upon the equilibria of reactions yielding an excess of H ions, and is not necessarily related to the various phenomena grouped under the terms "absorption" and "adsorption." Although the literature concerning soils constantly refers to "absorption" and "adsorption," yet no very concise meaning has been attached to these terms. In fact, there is quite as much evidence in favor of a chemical, as opposed to a physical, interpretation of such phenomena. For these reasons a more critical examination of this field would be a welcome addition to agricultural science.

DESCRIPTION OF EXPERIMENTAL APPARATUS

The apparatus used in determining the H-ion concentration is similar to that described by Hildebrand (17), with such modifications as are necessary or convenient for purposes of soil investigations (fig. 1). Precise methods for determining small differences of potential have frequently been described, but the use of an elaborate potentiometer system is neither practicable nor necessary in work with soils or nutrient solutions. Obviously measurements of physical-chemical exactitude are useless unless all the factors involved are capable of equally exact control, which is not possible with most substances of agricultural interest. For practical application, therefore, the voltmeter method of Hildebrand is entirely adequate in point of accuracy and at the same time rapid and convenient.

For information in regard to the physical-chemical principles underlying the method, the reader is referred to Hildebrand (17), Michaelis (21), Itano (18), or to textbooks on electrochemistry. Very convenient tables for the transformation of voltmeter readings into H- and OH-ion concentrations have been prepared by Schmidt (30). It is deemed desirable, however, to present here certain details of the apparatus and method of procedure, since these are not easily available to the general worker in agricultural laboratories. Moreover, measurements with soil suspensions require special precautions, to avoid otherwise very misleading results.

The arrangement of the apparatus and method of wiring are shown in figure 1. The entire system includes the following pieces of apparatus: Dry cell, two rheostats of 40 ohms resistance, with sliding contacts; Weston voltmeter, 0 to 1.2 volts; Leeds and Northrup portable galvanometer, sensitivity of 1 megohm; contact key; calomel cell, hydrogen-electrode vessel; hydrogen generator, with purifying tube, wash bottles, and rheostat or lamp board connected with direct current.

A convenient Cottrell hydrogen electrode, designed by the Department of Chemistry, University of California, consists of a glass tube of about 1 cm. in diameter and 15 cm. in length, in the end of which is sealed a

cylinder of fine platinum gauze approximately 1 cm. long. Near the upper end of the electrode a branch tube permits the entrance of hydrogen gas. A platinum wire is affixed to the gauze at one end and sealed into a small glass tube at the other. The electrical connection is then

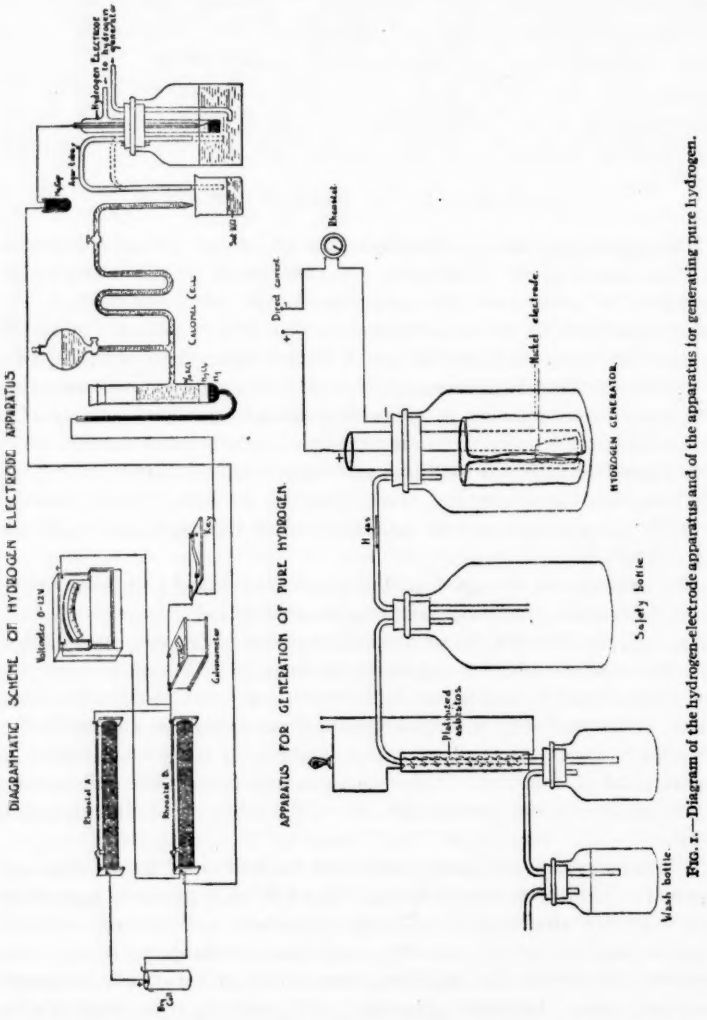


FIG. 1.—Diagram of the hydrogen-electrode apparatus and of the apparatus for generating pure hydrogen.

made by the use of a copper wire and a mercury cup, as shown in figure 1. The electrode must be gas-tight at the top. Platinum black is deposited on the gauze, as described by Ostwald (22). The coating should be renewed occasionally. It is sometimes necessary to clean the plat-

inum between determinations with a little diluted hydrochloric acid, afterward rinsing in many changes of distilled water.

The electrode vessel must be closed to the air. A simple and easily constructed cell may be made from a wide-mouthed bottle of 75 to 100 c. c. capacity. Through holes in the rubber stopper are fitted the hydrogen electrode, an agar connecting tube, a small exit tube, and a tube for saturating the solution with hydrogen when desired. A hole to admit the tip of a burette should also be provided for purposes of titration. The most convenient method of making the liquid connection is by means of bent glass tubes, filled with agar jelly prepared with a saturated solution of potassium chlorid. Only thoroughly washed agar should be used in making the jelly. In order to avoid contact potentials as far as possible connection with the calomel cell is made through a beaker, containing saturated potassium-chlorid solution. The construction of a normal calomel cell is described by Ostwald (22). In the present work *N*/10 potassium chlorid was used.

An adequate supply of pure hydrogen is of primary importance, and this is most conveniently obtained from the decomposition of water by means of a direct electric current. Such a generator may easily be prepared by using a large bottle and an inner cylinder made from a wide glass tube as a means of separating the electrodes. The latter may be of nickel, or pure iron and a 25 per cent (by weight) potassium hydroxid solution is convenient as an electrolyte. In order to purify the hydrogen from small quantities of oxygen, it is passed through a long glass tube filled with platinized asbestos. This is heated by a fine nichrome wire wound around the outside of the tube and connected through a lamp with a source of current. To provide a rapid stream of hydrogen requires the consumption of several amperes of current.

EXPERIMENTAL PROCEDURE

After placing the soil suspension in the bottle, hydrogen gas is permitted to flow through the electrode raised above the surface of the liquid for several minutes. The electrode is now lowered until the platinum gauze is partially submerged in the liquid and the exit tube closed. The bottle is now rotated back and forth for several minutes, as originally described by Hasselbach and Gammeltoft (16). The agar tube (at other times kept out of the liquid) is now lowered so that a connection is made with the calomel cell through the beaker of potassium chlorid. The rheostats are adjusted so that no deflection of the galvanometer needle is noted when momentary connection is made by tapping the key. The reading on the voltmeter is then recorded. The procedure is repeated until constant readings are obtained. This occurs in the case of acid soils within a few minutes, but for soils approximately neutral a slightly longer time will be required. In the case of titrations

prolonged shaking is required after each addition of the titrating solution, in order to obtain constant readings. This is due to the slow rate of solubility possessed by the acid constituents of the soil. The use of a mechanical shaking device would doubtless greatly facilitate the operation.

Duplicate determinations on soil suspensions usually agree within 0.01 to 0.02 volt. In any one determination constancy of readings may be obtained to within 0.002 volt. The larger sources of error result from the change in the decomposition of the solution due to reduction of nitrates or the breaking down of the HCO_3 ions. These points have already been thoroughly considered. Robertson (28) and later Desha and Acree (11) have shown the possible interference of certain types of organic matter. Saturation of the electrode with hydrogen before dipping seems to obviate the error, and no difficulty from this source was experienced in the present work.

The use of the modification of Hildebrand's apparatus described in this paper and due observance of the special precautions noted will, it is believed, enable the investigator of soils and plants to obtain valuable information in regard to H-ion concentrations without undue loss of time. In many fields of biochemistry [1] similar methods have been extensively employed during the last few years.

SUMMARY

(1) Soil acidity is due to the presence of an excess of hydrogen ions in the soil solution.

(2) Direct evidence of this fact is given by hydrogen-electrode measurements.

(3) The hydrogen-ion concentration of different soil suspensions was found to vary within wide limits, from a condition of high acidity to one of high alkalinity.

(4) Soils containing calcium in equilibrium with HCO_3^- and CO_2 have a very slightly alkaline reaction.

(5) The effect of heating, grinding, and of varying the ratio of soil to water on the hydrogen-ion concentration was studied.

(6) An electrometric method for the determination of the lime requirement of soils is suggested.

(7) The addition of sodium chlorid, potassium chlorid, and barium chlorid to certain soil suspensions was found to increase the hydrogen-ion concentration.

[1] For a review of the literature concerning the application of the hydrogen electrode to biochemistry the reader is referred to the following:

Schmidt, C. L. A. Changes in the H+ and OH⁻ concentration which take place in the formation of certain protein compounds. *In* Jour. Biol. Chem., v. 25, no. 1, p. 63–79, 9 fig. 1916. Bibliographical footnotes.

Sörensen, S. P. L. Über die Messung und Bedeutung der Wasserstoffionenkonzentration bei biologischen Prozessen. *In* Ergeb. Physiol., Jahrg. 12, p. 393–532, 12 fig. 1912. Literatur, p. 394–398.

(8) Several phases of the phenomena designated "adsorption" were studied, with special reference to the removal of OH ions by the soil from solutions of various hydrates.

(9) There appears to be a simultaneous removal of positive and negative ions from solution by soils.

(10) Some general theoretical considerations with regard to the relation of adsorption to chemical reactions in soils are presented.

(11) A convenient method of procedure for utilizing the hydrogen electrode in soil studies is described.

LITERATURE CITED

(1) AMES, J. W., and SCHOLLENBERGER, C. J.
1916. Comparison of lime requirement methods. *In* Jour. Indus. and Engin. Chem., v. 8, no. 3, p. 243–246.

(2) BILLITZER, Jean.
1903. Eine Theorie der Kolloide und Suspensionen. *In* Ztschr. Phys. Chem., Bd. 45, Heft 3, p. 307–330.

(3) BOGUE, R. H.
1915. The adsorption of potassium and phosphate ions by typical soils of the Connecticut Valley. *In* Jour. Phys. Chem., v. 19, no. 8, p. 665–695, 13 fig.

(4) BROWN, P. E., and JOHNSON, H. W.
1915. The effect of grinding the soil on its reaction as determined by the Veitch method. *In* Jour. Amer. Soc. Agron., v. 7, no. 5, p. 216–220.

(5) CAMERON, F. K.
1911. The Soil Solution . . . 136 p., 3 fig. Easton, Pa.

(6) ——— and BELL, J. M.
1907. The action of water and aqueous solutions upon soil carbonates. U. S. Dept. Agr. Bur. Soils Bul. 49, 64 p., 5 fig.

(7) CLARKE, F. W., and STEIGER, George.
1902. The action of ammonium chloride upon silicates. U. S. Geol. Survey Bul. 207, 57 p.

(8) CONNER, S. D.
1916. Acid soils and the effect of acid phosphate and other fertilizers upon them. *In* Jour. Indus. and Engin. Chem., v. 8, no. 1, p. 35–40, 2 fig.

(9) COOK, R. C.
1916. Effect of grinding on the lime requirement of soils. *In* Soil Science, v. 1, no. 1, p. 95–98.

(10) DAIKUHARA, G.
1914. Ueber saure Mineralböden. *In* Bul. Imp. Cent. Agr. Exp. Sta. Japan, v. 2, no. 1, p. 1–40, 1 pl.

(11) DESHA, L. J., and ACREE, S. F.
1911. On difficulties in the use of the hydrogen electrode in the measurement of the concentration of hydrogen ions in the presence of organic compounds. *In* Amer. Chem. Jour., v. 46, no. 6, p. 638–648, 1 fig.

(12) FISCHER, Gustav.
1914. Die Säuren und Kolloide des Humus. *In* Kühn Archiv, v. 4, p. 1–36, 4 fig. Literaturverzeichnis, p. 135–136.

(13) GILLESPIE, L. J.
1916. The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, p. 7–16, 2 fig.

(14) HANLEY, J. A.
1914. Estimation of the surface of soils. *In* Jour. Agr. Sci., v. 6, pt. 1, p. 58–62.
(15) HARRIS, J. E.
1914. Soil acidity. Mich. Agr. Exp. Sta. Tech. Bul. 19, p. 524–536.
(16) HASSELBACH, K. A., and GAMMELTOFT, S. A.
1915. Die Neutralitätsregulation des graviden Organismus. *In* Biochem Ztschr., Bd. 68, Heft 3/4, p. 206–264, 2 fig.
(17) HILDEBRAND, J. H.
1913. Some applications of the hydrogen electrode in analysis, research and teaching. *In* Jour. Amer. Chem. Soc., v. 35, no. 7, p. 847–871, 15 fig.
(18) ITANO, Arao.
1916. The relation of hydrogen ion concentration of media to the proteolytic activity of Bacillus subtilis. *In* Mass. Agr. Exp. Sta. Bul. 167, pt. 1, p. 139–177, illus.
(19) JOHNSON, John.
1916. The determination of carbonic acid, combined and free, in solution, particularly in natural waters. *In* Jour. Amer. Chem. Soc., v. 38, no. 5, p. 947–975.
(20) LOEW, Oscar.
1913. Studies on acid soils of Porto Rico. Porto Rico Agr. Exp. Sta. Bul. 13, 23 p., 1 fig.
(21) MICHAELIS, Leonor.
1914. Die Wasserstoffionenkonzentration. 210 p., 41 fig. Berlin. Literaturverzeichnis, p. 196–207.
(22) OSTWALD, W. F.
1910. Hand- und Hülfsbuch zur Ausführung physiko-chemischer Messungen. Aufl. 3, hrsg. von R. Luther und K. Drucker. 573 p., 351 fig. Leipzig.
(23) OSTWALD, Wolfgang.
1909. Grundriss der Kolloidchemie. 525 p., Dresden.
(24) PARKER, E. G.
1913. Selective adsorption by soils. *In* Jour. Agr. Research, v. 1, no. 3, p. 179–188, 2 fig.
(25) PATTEN, H. E.
1907. Energy changes accompanying absorption. *In* Trans. Amer. Electrochem. Soc., v. 11, p. 387–405, 1 fig. Discussion, p. 405–407.
(26) RICE, F. E.
1916. Studies on soils. I. Basic exchange. *In* Jour. Phys. Chem., v. 20, no. 3, p. 214–227, 1 fig.
(27) ROBERTSON, T. B.
1908. Einige kritische Bemerkungen zur Theorie der Adsorption. *In* Ztschr. Chem. und Indus. Kolloide, Bd. 3, Heft 2, p. 49–76.
(28) ———.
1910. Studies in the electrochemistry of the proteins. I. The dissociation of potassium caseinate in solutions of varying alkalinity. *In* Jour. Phys. Chem., v. 14, no. 6, p. 528–568, 2 fig.
(29) SAIDEL, Teodor.
1913. Quantitative Untersuchungen über die Reaktion wässeriger Bodenauszüge. *In* Bul. Sect. Sci. Acad. Roumaine, ann. 2, no. 1, p. 38–44, 3 fig.
(30) SCHMIDT, C. L. A.
1909. Table of H^+ and OH^- concentrations corresponding to electromotive forces determined in gas-chain measurements. *In* Univ. Cal. Pub. Phys., v. 3, no. 15, p. 101–113.

(31) SHARP, L. T.
1915. Salts, soil-colloids, and soils. *In* Proc. Nat. Acad. Sci., v. 1, no. 12, p. 563–568.

(32) SULLIVAN, E. C.
1907. The interaction between minerals and water solutions. U. S. Geol. Survey Bul. 312, 69 p.
Cites (p. 20–23) work by Lemberg.

(33) TRUOG, E.
1915. Soil acidity and methods for its detection. *In* Science, n. s. v. 42, no. 1084, p. 505–507.

(34) VAN BEMMELEN, J. M.
1888. Die Absorptionsverbindungen und das Absorptionsvermögen der Ackererde. Dritte Abhandlung. *In* Landw. Vers. Sta., Bd. 35, p. 69–136.

(35) VEITCH, F. P.
1904. Comparison of methods for the estimation of soil acidity. *In* Jour. Amer. Chem. Soc., v. 26, no. 6, p. 637–662.